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PRINCIPAL INVESTIGATOR: Wendell W. Weber, M.D., Ph.D.

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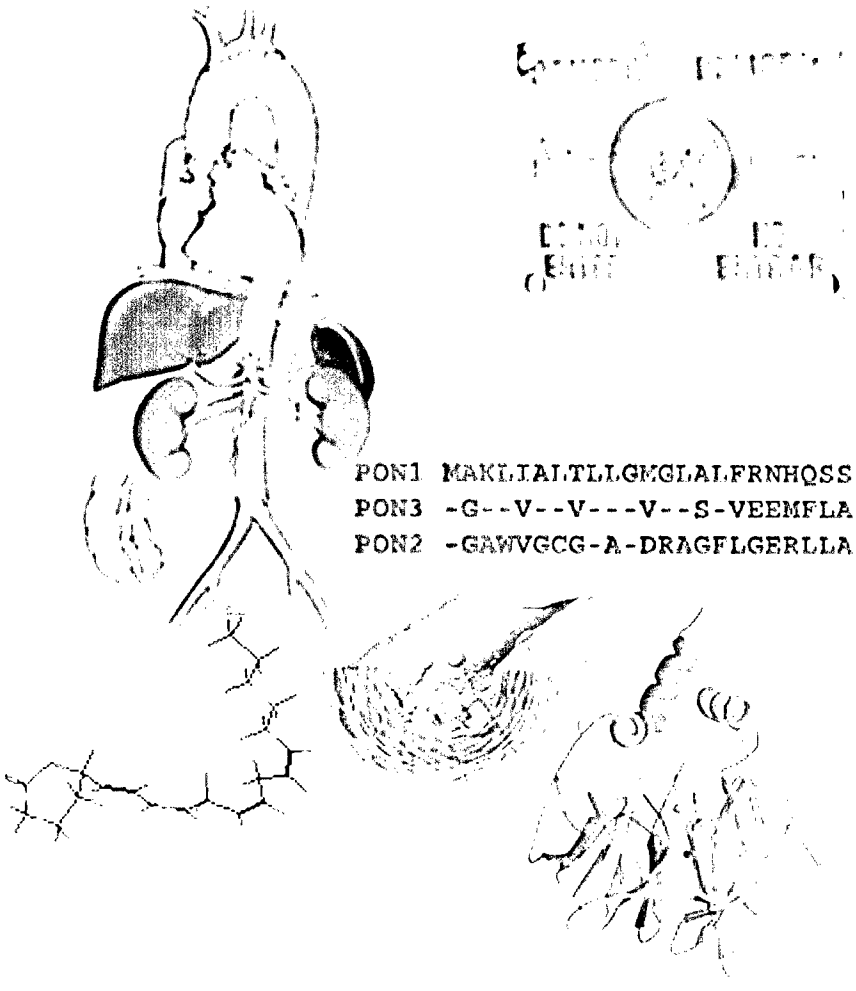
1st International Conference Paraoxonases

Basic and Clinical Directions of Current Research



PROGRAM and ABSTRACT BOOK

April 22-24 • 2004

Michigan Union, University of Michigan

Ann Arbor, Michigan, U.S.A.

Conference Sponsors

The Organizing Committee would like to acknowledge and thank the following organizations and companies for their generous support for this conference:

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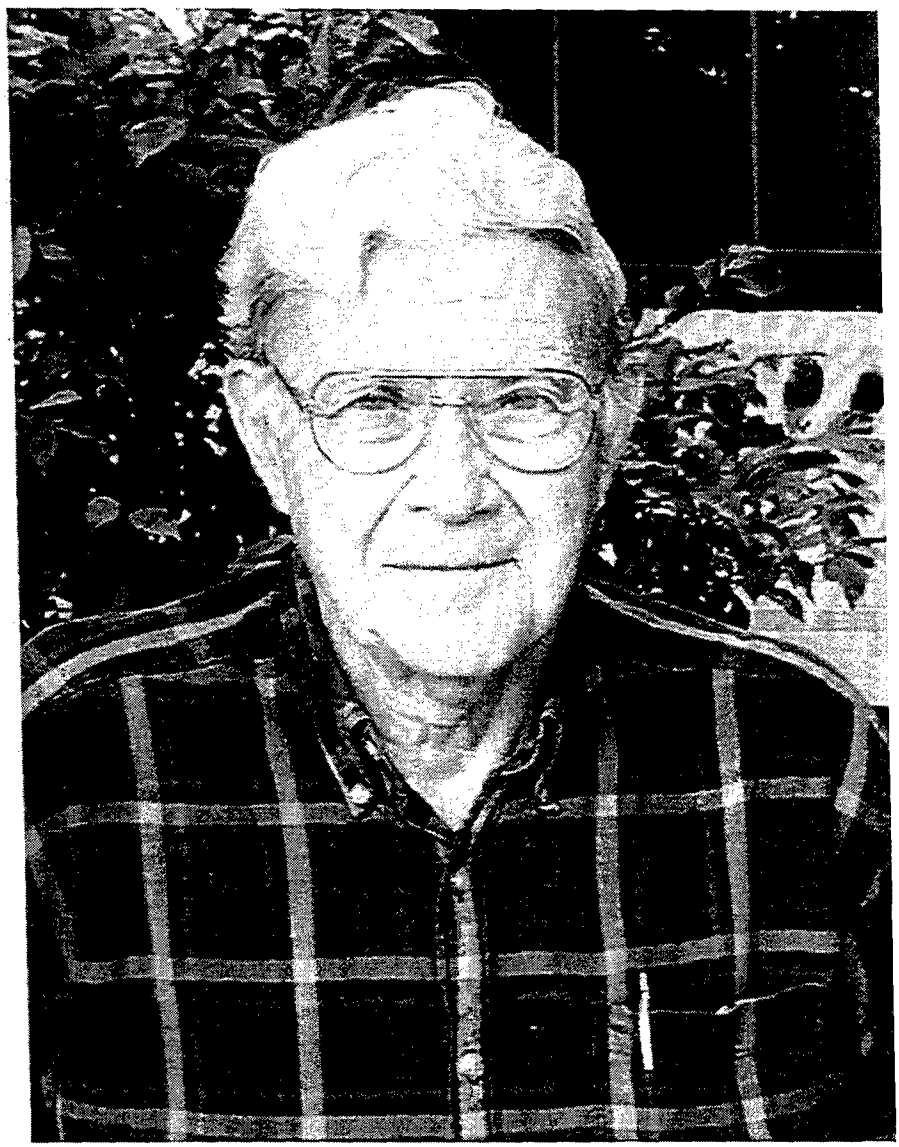

Prof. Emeritus Bert N. La Du, Jr., MD, Ph.D.

for his personal and scientific contributions to the paraoxonase research.

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Srinivasa Reddy, University of California, Los Angeles, CA, USA

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1st International Conference • Paraoxonases • Basic and Clinical Directions of Current Research

THURSDAY, APRIL 22, 2004

5:00 PM– 7:00 PM	Registration and Reception / Michigan Union, 1st Floor, Kuenzel Room

FRIDAY, APRIL 23, 2004

8:00 - 8:40 AM	Registration, Continental breakfast / Michigan Union, 1st Floor, Anderson Room
	Poster setup / Michigan Union, 1st Floor, Anderson and Pond Rooms
8:40 – 8:50 AM	Welcome and Opening Remarks / Michigan Union, 1st Floor, Kuenzel Room

FRIDAY, APRIL 23, 2004 – Morning Sessions/Michigan Union, 1st Floor, Kuenzel Room

I. GENERAL ASPECTS OF PON RESEARCH Session 1

Chairmen Bert La Du and Michael Mackness

8:50 – 9:15 Invited presentation

Evolution and Phylogenetic Relationships of the Paraoxonases

Bert N. La Du, University of Michigan, Ann Arbor, USA

9:15 – 9:40 Invited presentation

Enzymatic Activities and Substrate Specificity for PONs 1, 2 and 3

Dragomir Draganov, University of Michigan, Ann Arbor, USA

9:40 – 9:55 Selected Posters Presentations

Application of the Integrated Michaelis Equation to the Study of Paraoxonase

Thierry Dantoine, Dupuytren University Hospital, Limoges, France

A Fluorogenic Substrate for Detection of Organophosphatase Activity.

Serguei Soukharev, American Red Cross, Rockville, USA

9:55 AM – 10:25 AM Coffee and tea, Poster Viewing / Michigan Union, 1st Floor, Anderson and Pond Rooms

I. GENERAL ASPECTS OF PON RESEARCH Session 2

Chairmen Oksana Lockridge and Dragomir Draganov

10:35 – 11:00 Invited presentation

Human Serum Paraoxonase (PON1): Structure-Function Relationships

Brian J. Bahnson, University of Delaware, Newark, USA

11:00 – 11:15 Short Communication

Directed Evolution of Paraoxonases PON1 and PON3 for Bacterial Expression and Catalytic Specialization

Amir Aharoni, The Weizmann Institute of Science, Rehovot, Israel

11:15 – 11:40 Invited presentation

The 3D-Structure, Mechanism and Evolution of Serum Paraoxonases

Joel Sussman and Dan Tawfik, The Weizmann Institute of Science, Rehovot, Israel

11:40 AM – 11:55 AM Selected Posters Presentations

X-ray Structure of an Unknown Protein Co-purified with Human Paraoxonase

Eric Chabrier, CNRS-Université Henri Poincaré, Vandoeuvre-lès-Nancy, France

Structure/Function Analyses of Human Paraoxonase-1 Mutants

David T. Yeung, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds, USA

11:55 AM – 1:00 PM Lunch Break / Michigan Union, 1st Floor, Anderson Room

FRIDAY, APRIL 23, 2004 - Afternoon Sessions/Michigan Union, 1st Floor, Kuenzel Room

II. PONs GENES, EXPRESSION AND REGULATION

Chairmen Wendell W. Weber and Clement Furlong

1:00 PM – 1:25 PM Invited presentation

Functional Genomics of PON1.

Clement Furlong, University of Washington, Seattle, USA:

1:25 PM – 1:50 PM Invited presentation

The Impact of Genetic and Non-Genetic Factors on Serum Paraoxonase Activity

Richard W. James, University Hospital, Geneva University, Geneva, Switzerland.

1:50 PM – 2:05 PM Short communication

VLDL: An Alternative Vector for Paraoxonase 1 Secretion

Sara Deakin, Geneva University, Geneva, Switzerland

2:05 PM – 2:30 PM Invited presentation

Attempts to Define the Biologic Function of Paraoxonases

Srinivasa Reddy, UCLA, Los Angeles, USA

2:30 PM – 2:45 PM Short Communication

Oxidative Stress Induces Expression of Paraoxonase 2 (PON2)

Natividad Villa, UCLA, Los Angeles, USA

2:45 PM – 3:00 PM Short Communication

Paraoxonase 2 Expression Is Up-regulated by NADPH Oxidase during Monocyte Differentiation into Macrophages

Maayan Shiner, Rambam Medical Center, Haifa, Israel

3:00 PM – 3:20 PM Selected Posters Presentations

Functional Polymorphisms and Triglycerides Are Independent Determinants of Pon1 Enzymatic Activity in Pregnant Women

James Wetmur, Mount Sinai School of Medicine, New York, USA.

Anxiety Scores in the HERITAGE Family Study Associate with Expression Variabilities and Polymorphisms in the Acetylcholinesterase / Paraoxonase Locus

Ella Sklan, Hebrew University of Jerusalem; Jerusalem, Israel

Intestinal paraoxonases: Possible role against oxidative stress

Raanan Shamir, Meyer Children's Hospital of Haifa, Haifa, Israel

3:20 PM – 3:45 PM Coffee and tea, Poster Viewing / Michigan Union, 1st Floor, Anderson and Pond Rooms

III. ROLE OF PON1 IN ORGANOPHOSPHATE TOXICITY

Chairmen Clarence Broomfield and Lucio Costa

3:45 PM – 4:10 PM Invited presentation

Role of paraoxonase in organophosphate toxicity

Lucio G. Costa, University of Washington, Seattle, WA, USA

4:10 PM – 4:35 Invited presentation

New Toxicological Aspects of Paraoxonase

Bharti Mackness, University of Manchester, Manchester, UK:

4:35 PM – 5:00 PM Invited presentation

Neuroepidemiologic Requirements for Detecting Associations of PON1 Isoenzyme Activity with Gulf War Illness

Robert Haley, University of Texas Southwestern Medical Center, Dallas, USA

5:00 PM – 5:15 PM Short Communication

Improving catalytic properties of recombinant PON1 toward detoxification of organophosphate compounds by in vitro evolution

Leonid Gaydukov, The Weizmann Institute of Science, Rehovot, Israel

5:15 PM – 5:30 PM Selected Posters Presentations

Recombinant Organophosphorus Acid Anhydrolase (OPH, Paraoxonase) as an Active Therapeutic Agent Derived from Nanotechnology

Ilona Petrikovics, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, USA

The Relationship between PON1 Q192R Genotype and Phenotype in Human Liver

Eleine Mutch, University of Newcastle upon Tyne, Tyne and Wear, UK

7:00 – 9:30 PM Dinner/Banquet. Honouring of Prof. B.N. La Du / Michigan League, 2nd Floor, Vandenberg Room

Bert N. La Du – 50 Years of Research and Counting...

Wendell W. Weber, University of Michigan, Ann Arbor, USA

SATURDAY, APRIL 24, 2004

8:00 AM - 8:30 AM Continental breakfast, Poster visitation/Michigan Union, 1st Floor, Anderson and Pond Rooms

SATURDAY, APRIL 24, 2004 – Morning Sessions / Michigan Union, Kuenzel Room

IV. PONs AND ATHEROSCLEROSIS – Session 1

Chairmen Michael Aviram and Mohamad Navab

8:30 AM – 8:55 AM Invited presentation

Paraoxonase and Atherosclerosis: Is the Gene or the Protein More Important?

Michael Mackness, University of Manchester, Manchester, UK

8:55 AM – 9:20 AM Invited presentation

Paraoxonase (PON 1) Protects Against Lipids Peroxidation and Attenuates Atherosclerosis Development

Michael Aviram, Rambam Medical Center, Haifa, Israel

9:20 AM – 9:35 AM Short Communication

HDL-associated PON1 Enhances HDL-mediated Cholesterol Efflux from Macrophages via the ABCA1 Transporter: a Possible Role for Lysophosphatidylcholine

Mira Rosenblat, Rambam Medical Center, Haifa, Israel

9:35 AM - 9:50 AM Short Communication

Paraoxonase genotype and phenotype contribute to plasma high-density lipoprotein levels in familial hypercholesterolemia

Thomas Van Himbergen, UMC Utrecht, Utrecht, The Netherlands

9:50 AM – 10:05 AM Short Communication

Decreased protection by HDL from elderly subjects against LDL oxidation: role of Paraoxonase antioxidant activity

Abdelolahead Khalil, University of Sherbrooke, Sherbrooke, Canada

10:05 AM – 10:15 AM Selected poster presentation

Study of factors influencing the decreased paraoxonase activity with aging

Idiko Seres, University of Debrecen, Debrecen, Hungary

10:15 AM – 10:45 AM Coffee and tea, Poster Viewing / Michigan Union, 1st Floor, Anderson and Pond Rooms

IV. PONs AND ATHEROSCLEROSIS – Session 2.

Chaimen Trude Forte and Srinivasa Reddy

10:45 AM – 11:10 AM Invited presentation

PON1: Its Role in Athero-protection in Mice

Trudy Forte, Lawrence Berkley National Laboratories, Berkley, USA

11:10 AM – 11:35 AM Invited presentation

The functions of PON1 and PON3: studies of transgenic mouse models

Diana Shih, UCLA, Los Angeles, USA

11:35 AM – 12:00 PM Invited presentation

ApoA-I mimetic peptide forms HDL like particles containing paraoxonase 1 in apo E null mice

Mohamad Navab, UCLA, Los Angeles, USA:

12:05 PM – 1:00 PM Lunch Break / Michigan Union, 1st Floor, Anderson Room

IV. PONs AND ATHEROSCLEROSIS – Session 3

Chairmen Richard James and Philip Connelly

1:00 PM – 1:25 PM Invited presentation

The Role of Apolipoprotein AI and Paraoxonase 1 in the Formation of Bioactive Phospholipid Oxidation Products

Philip Connelly, University of Toronto, Toronto, Canada:

1:25 PM – 1:40 PM Short Communication

Purified Human Serum PON1 Does Not Protect LDL against Oxidation in the *in vitro* Assays Initiated with Copper or AAPH

John Teiber, University of Michigan, Ann Arbor, USA

1:40 AM – 1:55 PM Short Communication

Homocysteine-thiolactonase Activity and PON1 Genotype in Coronary Artery Disease

Heronim Jakubowski, New Jersey Medical School, Newark, USA

1:55 PM – 2:10 AM Short Communication

Role of paraoxonase1 (Q192R) polymorphism on total body fat in healthy volunteers

Ahim Bub, Institute of Nutritional Physiology, Karlsruhe, Germany

2:10 PM – 2:40 PM Selected poster presentations

Effect of extended-release fluvastatin on serum paraoxonase activity

Gyorgy Parah, University of Debrecen, Debrecen, Hungary

The effects of paraoxonase on cholesterol lowering by simvastatin and atorvastatin

Mark Roest, UMC Utrecht, Utrecht, The Netherlands

Population-based and family studies suggest an association between PON1 Gln192Arg polymorphism and premature coronary heart disease in female Mauritians of Indian origin

Meera Manraj, University of Mauritius, Moka, Mauritius

Paraoxonase and superoxide dismutase gene polymorphisms in Italian patients with noise-induced hearing loss

Guliana Fortunato, Universitá Federico II di Napoli, Napoli, Italy

2:40 PM – 3:10 PM Coffee and tea, Poster Viewing/ Michigan Union, 1st Floor, Anderson and Pond Rooms

V. ROUND TABLE DISCUSSIONS

3:10 PM – 3:55 PM **PONs in clinical studies**

Moderators Richard James and Michael Aviram

3:55 PM – 4:15 PM **PONs nomenclature**

Moderators Dragomir Draganov and Srinivasa Reddy

4:15 PM Closing Remarks

POSTERS (* selected poster presentation) / Michigan Union, Anderson and Pond Rooms

1. **Effects of some drugs on enzyme activities of paraoxonase 1 (PON1) in human serum in vitro**
   Hatice BOZKURT, Balikesir University, Balikesir, Turkey

2. **Interacting ACHE and PON1 polymorphisms modulate plasma acetylcholinesterase and paraoxonase activities**
   Boris BRYK,Hebrew University of Jerusalem, Jerusalem, Israel

3. **X-ray structure of an unknown protein co-purified with human paraoxonase***
   Eric CHABRIERE, Université Henri Poincaré, Nancy, France

4. **Paraoxonase 1 gene (PON1) polymorphisms and human sperm quality**
   Yahya DAHMANI,Universidad de Zaragoza, Zaragoza, Spain

5. **Application of the integrated Michaelis equation to the study of paraoxonase***
   Thierry F. DANTOINE, Dupuytren University Hospital, Limoges, France

6. **High level expression and purification of human paraoxonases using a baculovirus system**
   Dragomir DRAGANOV, University of Michigan, Ann Arbor

7. **Paraoxonase and superoxide dismutase gene polymorphisms in Italian patients with noise-induced hearing loss***
   Giuliana FORTUNATO, Universitá Federico II di Napoli, Napoli, Italy

8. **Low Type Q Paraoxonase/Arylesterase in Ill Gulf War Veterans Not Due to Circulating Proinflammatory Cytokines**
   Robert W. HALEY, University of Texas Southwestern Medical Center, Dallas, USA

9. **Paraoxonase genotype and phenotype contribute to plasma high-density lipoprotein levels in familial hypercholesterolemia***
   Thomas van HIMBERGEN, UMC Utrecht, Utrecht, The Netherlands

10. **Study of serum paraoxonase activity from brain tumour patients in Turkey**
    Feray KOCKAR, Balikesir University, Balikesir, Turkey

11. **The difference of human and pig serum arylesterase in the hydrolysis of different para-substituted phenyl acetate substrates**
    Fei LIAO, Chongqing University of Medical Sciences, Chongqing, P. R. China

12. **Population-based and family studies suggest an association between PON1 Gln192Arg polymorphism and premature coronary heart disease in female Mauritians of Indian origin***
    Meera MANRAJ,University of Mauritius, Moka, Mauritius

13. **Reduction of Serum Paraoxonase Activities in Mexican Stroke Patients**
Antonio MONROY-NOYOLA, Universidad Autónoma del. Estado de Morelos, México

14. **The relationship between PON1 Q192R genotype and phenotype in human liver***
Elaine MUTCH, University of Newcastle upon Tyne, Tyne and Wear, UK

15. **PON1 variant frequencies in a population-based sample of children**
Susan S. NIELSEN, University of Washington; Seattle, USA

16. **Polymorphic variation in PON1 affects the detoxification of diazoxon in human serum**
Karen A.O'LEARY, Imerial College London, London, UK

17. **Effect of extended-release fluvastatin on serum paraoxonase activity***
Gyorgy PARAGH, University of Debrecen, Debrecen,Hungary

18. **Recombinant Organophosphorus Acid Anhydrolase (OPH, Paraoxonase) as an Active Therapeutic Agent Derived from Nanotechnology***
Ilona PETRIKOVICS, U.S. AMRICD, *Aberdeen Proving Ground, USA*

19. **The effects of paraoxonase on cholesterol lowering by simvastatin and atorvastatin***
Mark ROEST, UMC Utrecht, Utrecht, The Netherlands

20. **Study of factors influencing the decreased paraoxonase activity with aging***
Ildiko SERES, University of Debrecen, Debrecen, Hungary

21. **Intestinal paraoxonases. Possible role against oxidative stress***
Raanan SHAMIR, Meyer Children's Hospital of Haifa, Haifa, Israel

22. **Differential effects of some drugs on serum and liver paraoxonase activity in mouse**
Selma SINAN, Balikesir University, Balikesir, Turkey

23. **Anxiety Scores in the HERITAGE Family Study Associate with Expression Variabilities and Polymorphisms in the Acetylcholinesterase / Paraoxonase Locus**
Ella H. SKLAN, Hebrew University of Jerusalem, Jerusalem, Israel

24. **A fluorogenic substrate for detection of organophosphatase activity**
Serguei SOUKHAREV, American Red Cross, Rockville, USA

25. **Functional Polymorphisms and Triglycerides Are Independent Determinants of Pon1 Enzymatic Activity in Pregnant Women***
James G.WETMUR, Mount Sinai School of Medicine, New York, USA

26. **Structure/Function Analyses of Human Paraoxonase-1 Mutants***
David T.YEUNG, U.S. AMRICD, Aberdeen Proving Grounds, USA

PRESENTING AUTHORS INDEX

AHARONI, Amir *Short Communication, Friday, April 23, 2004, 11:00 – 11:15 AM*

Directed evolution of paraoxonases PON1 and PON3 for bacterial expression and catalytic specialization

Amir Aharoni, Leonid Gaidukov, Dan S. Tawfik
Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Despite their key roles in organophosphate (OP) detoxification and in preventing atherosclerosis, the structure and mechanism of PONs are poorly understood. PONs seem like "Jacks of all trades", acting on a very wide range of substrates most of which are of no physiological relevance. Family shuffling and screening lead to the first PON variants that express in a soluble and active form in *E. coli* at >30 mg/ L culture. We describe variants, of both PON1 and PON3 with kinetic parameters similar to those reported for PONs purified from sera, and others that show dramatically increased activities. In particular, we have evolved PON1 variants with OP-hydrolyzing activities 40-fold higher than wild-type and a specificity switch of >2000 fold, producing PONs specialized for OP rather than ester hydrolysis. More recent work aims at the directed evolution of new PON1 variants with increased homocysteine lactonase and lipase activities. Analysis of the newly-evolved variants provides insights into the structure and function of PONs, and into the evolutionary relationships between different family members.

AVIRAM, Michael *Invited Presentation, Saturday, April 24, 2004, 8:55 – 9:20 AM*

Paraoxonase (PON 1) Protects Against Lipids Peroxidation and Attenuates Atherosclerosis Development

Michael Aviram
Rambam Medical Center, Haifa, Israel

Abstract: Human serum paraoxonase (PON1) is physically associated with high density lipoprotein (HDL) and the activity of PON1 was shown to be inversely associated with the risk for atherosclerosis development (which is enhanced under oxidative stress). We thus questioned whether this is related to the ability of PON 1 to protect against lipids peroxidation in lipoproteins and in arterial cells. Results: PON1 hydrolyzed cholesteryl linoleate hydroperoxides (CL-OOH) in oxidized LDL, HDL and macrophages and in human and mice atherosclerotic coronary or carotid lesions, yielding in the formation of linoleic acid hydroperoxides (L-OOH) and linoleic acid hydroxide (L-OH). This indicates esterase- and peroxidse-like activities for PON1. The PON1Q isoform was 50% more potent than PON1R in this respect. Using site-directed mutagenesis technique, the PON1's free sulfhydryl group at cysteine – 284 was found to be the enzyme active site. Macrophage foam cell formation is the hallmark of early atherogenesis and hence we questioned the effect of PON1 on macrophage atherogenicity. PON1 hydrolyzed macrophage lipid peroxides (PD) resulting in a 40% decreased content of PD, which was associated with a 36% reduced macrophage-mediated oxidation of LDL and also in a 44% decreased cellular uptake of oxidized LDL (secondary to a decrease in the expression of the scavenger receptor CD-36). On using PON1-deficient mice (PON1°/E°), a 42% increased atherosclerotic lesion size was shown in comparison to control E° mice and this was associated with increased (40-60%) oxidative stress in their serum, as well as in their macrophages (peritoneal and arterial). In macrophages from PON1° mice, NADPH oxidase activation was evident, resulting in enhanced cell-mediated oxidation of LDL. PON 1 action on macrophages reversed cellular oxidative stress toward normal levels.

Conclusion: PON1 anti-atherogenicity could be related to its ability to hydrolyze and neutralize specific atherogenic lipid peroxides in lesion's lipoproteins and arterial macrophages.

Biographical Sketch: Prof. Michael Aviram received his D.Sc. (Doctor of Sciences) degree in Biochemistry, at the Technion Faculty of Medicine, Haifa, Israel. Following two years (1978-1980) of a postdoctoral training at the Arteriosclerosis Center, M.I.T., Cambridge, MA, he was appointed as the Head of the Lipid Research Laboratory, Technion Faculty of Medicine, the Rappaport Institute for Research in the Medical Sciences and Rambam Medical Center, Haifa, Israel. Professor Aviram was a Visiting Scientist at the Center for Atherosclerosis Research, Columbia University, N.Y. (1986-1987) and a Visiting Associate Professor at the Department of Medicine, Division of Metabolism and Nutrition, University of WA, Seattle,WA (1987-1988). In 1997-1998 Aviram was a Visiting Professor at the University of Michigan Medical School, Ann Arbor, MI. Prof. Aviram's research focuses on the mechanism responsible for LDL oxidation as related to macrophage foam cell formation

and atherosclerosis. His unique contribution to this field is the elucidation of the roles of pro-oxidants and of anti-oxidants, which are associated with LDL and with arterial cells, in macrophage-mediated oxidation of LDL and atherosclerosis. Anti-oxidants studies include lipoprotein- and cell-associated flavonoids (from pomegranate, red wine, ginger and licorice), vitamin E, carotenoids (such as β-carotene and tomato's lycopene), as well as the macrophage glutathione system. The role of paraoxonases (PON) in protection against oxidative stress and atherosclerosis is also studied in Aviram's laboratory. Methodologies in use in the lab include cell culture, animal models for atherosclerosis (apo E and PON knockout mice), and human nutritional and pharmacological intervention studies. Prof. Aviram published over 250 original papers. In 1994 he was awarded in France, the Pasteur Institute Senior Investigator ARCOL International Prize for his research on lipoprotein oxidation, antioxidants and atherosclerosis, and in 1998 he received in Canada the Pfizer lecturer award.

BAHNSON, Brian J. *Invited Presentation, Friday, April 23, 2004, 10:35 – 11:00 AM*

Human Serum Paraoxonase (PON1): Structure-Function Relationships

Brian J. Bahnson[1], A. Khanal[1], C. McAndrew[1], D. Josse[2], D.T. Yeung[2], S.D. Kirby[2], J. Nicholson[2], and D.M. Cerasoli[2]

[1]Department of Chemistry & Biochemistry University of Delaware, Newark, USA
[2]Pharmacology Branch - U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds, Maryland, USA

Abstract: HuPON1 has been shown to exhibit various functional properties: esterase, anti-oxidative, phospholipid-binding and possible interaction with protein partners. However, the structural basis (catalytic sites, binding sites) and mechanisms underlying these properties have not been determined. Our works are aimed at establishing a homology model of the HuPON1 structure to help guide the interpretation of mechanism and even the specificity of interaction of HuPON1 with high-density lipoproteins. The model has been tested and found to be predictive by site-directed mutants of both structural and catalytic amino acid positions. These results are of importance in view of rationally engineering HuPON1, i.e., designing mutations to improve or create functional properties and to optimize the expression of a stable and active recombinant enzyme.

Biographical Sketch:
B.S.: Chemistry and Biochemistry double degree, cum laude, University of Massachusetts, Amherst, MA.
Ph.D: Chemistry, mentor: Vernon E. Anderson, Brown University, Providence, RI,
1990-1993: NIH Postdoctoral Fellow, mentor Judith P. Klinman, Dept. of Chemistry, University of California, Berkeley, CA.
1994: Visiting Assistant Professor of Chemistry, Williams College, Williamstown, MA.
1994-1998: Postdoctoral Associate, mentors Gregory A. Petsko and Dagmar Ringe, Rosenstiel Center, Brandeis University, Waltham, MA.
1998-present: Assistant Professor, Department of Chemistry & Biochemistry, University of Delaware, Newark, DE.

BOZKURT, Hatice *Poster*

Effects of some drugs on enzyme activities of paraoxonase 1 (PON1) in human serum in vitro

Hatice Bozkurt[1], Selma Sinan[1], Feray Kockar[1], Oktay Arslan[2], Nahit Gencer[2]
[1]Faculty of Science and Literature, [2]Department of Biology, Balikesir University, Balikesir, Turkey

Paraoxonase-1 (aryldialkylphosphatase, EC 3.1.8.1) (PON1) is a protein of 354 aminoacids with a molecular mass of 43 kDa. Mature PON1 retains its N-terminal hydrophobic signal sequence, which may be needed for binding to HDL. Human serum and liver paraoxonase has been shown to be important in the metabolism. In recent years, several lines of evidence have indicated that PON1, and perhaps other mammalian paraoxonases, act as important guardians against cellular damage from toxic agents, such as organophophates, oxidized lipids in the plasma low density lipoproteins (LDL) and against bacterial endotoxins.

In this study, the effects of cefamezin, ampisid, genta and magnesium sulfate on human serum paraoxonase (EC 3.1.8.1) have been investigated *in vitro*. Human serum paraoxonase was separately purified by ammonium sulfate precipitation and hydrophobic interaction chromatography. The enzyme activity was determined by Gan et al's method using paraoxan as a substrate. Inhibition effects of four different drugs on paraoxonase were determined using % esterase activity by plotting concentration of drugs. I_{50} values of the drugs exhibiting inhibition effects were found by means of these graphs. It was obserwed cefamezin, ampisid, genta and magnesium sulfate showed inhibition effect on paraoxonase activity.

This study was carried out at Balikesir University, Research Centre of Applied Sciences (Butam), Turkey.

BRYK, Boris *Poster*

Interacting ACHE and PON1 polymorphisms modulate plasma acetylcholinesterase and paraoxonase activities

Boris Bryk, Liat Ben-Moyal, Arik Eisenkraft, Shai Luria, Amir Cohen, Yoav Yehezkelli, Ariel Hourvitz, Hermona Soreq
Department of Biological Chemistry, Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem, Israel

The 5.5 Mb *ACHE/PON1* locus at chromosome 7q21-22 harbors two genes involved with organophosphate (OP) interactions. The acetylcholinesterase gene *ACHE* produces the acetylcholine hydrolyzing, OP-inhibitable AChE protein and is subject to exposure- or stress-induced overexpression, whereas the paraoxonase gene *PON1*, yields the OP-hydrolyzing PON enzyme which participates in protection from atherosclerosis and displays arylesterase activity. Both genes include biochemically effective genetic polymorphisms, but potential interactions between their effects remained unexplored. To address this issue, we genotyped seven polymorphic sites at the *ACHE/PON1* locus and determined the hydrolytic activities of the corresponding serum enzymes and of the AChE-related butyrylcholinesetrase (BChE) in 157 healthy Israelis. Plasma enzyme activities displayed 5.4, 3.3, 15.5 and 6.5-fold variability for AChE, BChE, PON and arylesterase activities, respectively. Average activities differed for carriers of distinct compound polymorphisms within the corresponding genes. Moreover, PON but not BChE or arylesterase activities displayed an inverse relationship with AChE, suggesting that low PON activities are associated with constitutive AChE overproduction. Our findings demonstrate that polymorphism(s) in the adjacent *PON1* and *ACHE* genes affect each other's expression, attribute a role in chemical protection to the multi-megabase *ACHE/PON1* cluster and predict for carriers of biochemically debilitating *ACHE/PON1* polymorphisms adverse genome-environment interactions.

Role of paraoxonase1 (Q192R)polymorphism on total body fat in healthy volunteers

Achim Bub, Stephan W. Barth, G. Rechkemmer
Federal Research Centre for Nutrition; Institute of Nutritional Physiology, Karlsruhe, Germany

PON1 polymorphisms may play a role in the development of impaired glucose tolerance and have an impact on the cardiovascular risk in patients with typ 2 diabetes. The aim of the present study was to investigate in healthy non-smoking male volunteers (n=24; 21-45y) the impact of the PON1 Q192R polymorphism on body fat and parameters of the lipid and glucose metabolism. The PON1 Q192R genotype was determined by RFPL-PCR. We measured PON1 activity, glucose, triacylglycerol, cholesterol, leptin, insulin, and plasminogen-activator-inhibitor type 1 (PAI-1) in serum. The QQ allele was detected in 13 volunteers, 9 subjects were heterozygous (QR) and 2 were homozygous for the mutation (RR). For statistical reasons we merged the R allele carrier (QR / RR). As expected, PON activity was higher in R allele carrier as compared to QQ subjects ($p<0.001$). Additionally, we found increased body fat, leptin and PAI-1 in R allele carriers ($p<0.05$). Triacylglycerol ($p=0.099$), insulin ($p=0.16$) and insulin resistance (HOMA: 1.76 ± 1.1 vs. 1.24 ± 0.65; $p=0.17$) tended to be higher in R allele carrier as compared to QQ volunteers. Serum glucose, total LDL and HDL cholesterol were not significantly different among these two groups. Body fat significantly correlated with leptin ($r=0.715$, $p<0.001$) and PAI-1 activity ($r=0.668$, $p<0.001$). Furthermore, PON1 activity correlated with leptin ($r=0.449$, $p=0.027$) and PAI-1 activity ($r=0.379$, $p=0.067$). In healthy men the PON1 Q192R polymorphism is associated with body fat and adipocyte related changes in leptin and PAI-1. The corresponding metabolic changes may point to impaired glucose tolerance in R allele carrier. Whether PON plays a causal role in the development of obesity and subsequent impaired glucose tolerance needs further investigation. The association of PON, leptin and PAI-1 may be interpreted in terms of a *linkage disequilibrium* since these genes are located on human chromosome 7.

CHABRIERE, Eric

Selected Poster Presentation, Friday, April 23, 2004, 11:40 –11:45 AM

X-ray structure of an unknown protein co-purified with human paraoxonase

Eric Chabriere, R. Morales, C. Contreras Martel, M. Nicodeme, F. Renault, M.L. Chesne, P. Carpentier, J.L. Ferrer, A. Fokine, J.C. Fontecilla-Camps, D. Rochu, P. Masson
LCM3B, Univeristé Henri Poincaré, Nancy, France.

Human paraoxonase (PON1, EC 3.1.8.1) is a plasma hydrophobic glycoprotein of 354 residues capable of hydrolyzing organophosphate (OP) insecticides and nerve agents. Thought no physiological function has been assigned to this enzyme, it appears to play a role against the development of atherosclerosis. The knowledge of the three-dimensional structure of PON1 is of the utmost importance to understand the catalytic mechanism of PON1. It is also a prerequisite for the rational design (site-directed mutagenesis) of PON1 mutants having an improved catalytic efficiency against nerve agents.

In order to solve the 3D structure of PON1 by X-ray crystallography, we have been undertaking to crystallize this enzyme. Three years ago we got crystals. After many efforts, *ab initio* calculations led to a low-resolution picture of the protein (Fokine, 2003), and phasing was possible after we obtained a heavy atom derivative. To our great surprise, the PON1 sequence did not fit into the electronic density map calculated with experimental phases at 1.8 Å. The structure solved was not PON1 but a totally unknown protein co-purified with PON1. Because the molecular weight of this protein is 38 KDa, it was not possible to discriminate from PON1 on SDS gels. This protein present at varying concentration in PON1 preparation may be associated to PON1 *in vitro*. The characterization of this new lipoprotein affords new insights into the PON1 physiological function(s). So far, the existence of this co-purified protein could explain the fail of PON1 crystallization attempts. For this reason, we are developing a new protocol of purification to obtain absolutely pure PON1.

Human PON1 and diisoprolylfluorophosphatase (DFPase) from *Loligo vulgaris* may have the same active site and folding. The 3D structure of DFPase has been solved (Scharff, 2001). Because diffraction data exist up to 0.8 Å resolutions, we refined this structure with MOPRO. This program allowing multipolar refinement, we hope to describe the active site with accuracy, locating electronic orbital. This work should provide a better understanding of catalytic mechanism of both DFPase and PON1.

CONNELLY, Philip *Invited Presentation, Saturday, April 24, 2004, 1:00 – 1:25 PM*

The Role of Apolipoprotein AI and Paraoxonase 1 in the Formation of Bioactive Phospholipid Oxidation Products

Philip Connelly
University of Toronto, Toronto, Ontario, Canada

Abstract: Paraoxonase-1 (PON1) is an enzyme that is associated with high density lipoproteins (HDL). We have carried out *in vitro* oxidation of HDL and apolipoprotein AI – phospholipid – PON1 complexes using 3-morpholinosydnonimine (SIN1) as a source of peroxynitrite. Phosphatidylcholine oxidation products were measured using LC-ESI-MS or ESI-MS-MS. We observed five major classes of oxidation product, hydroperoxides, beta-scission oxidation products (core aldehydes and core acids), hydroxides, isoprostanes and lysophospholipids. PON1 preparations had no effect on the accumulation of glycerophosphorylcholine hydroperoxides, hydroxides or isoprostanes. There was a significant reduction in beta-scission products and an increase in lysophosphatidylcholine. The phospholipase activity has now been shown by Marathe et al. (J Biol Chem 2003;278:3937), and confirmed by us, to be due to a minor component in the PON1 preparations. This component has properties similar to platelet activating factor acetyl hydrolase. When this factor is removed from the PON1 preparations, PON1 has no effect on oxidation of phospholipids by SIN1. These data will be compared with literature reports that PON1 reduces the concentration of lipid peroxides. It will be shown that, at present, there is no conclusive evidence that PON1 has an "anti-oxidant" activity towards lipoprotein phospholipids.

Biographical sketch:
Dr. Philip W. Connelly received his Ph.D. from the University of Toronto in 1983. He is currently a Staff Scientist at St. Michael's Hospital where he directs the J. Alick Little Lipid Research Laboratory. He is also an Associate Professor in the Departments of Medicine, Biochemistry and Laboratory Medicine and Pathobiology at the University of Toronto. Current research in the laboratory includes the study of cardiovascular risk factors in the Sandy Lake Oji-Cree and the biochemical and functional aspects of the oxidation of high density lipoproteins.

COSTA, Lucio G. *Invited Presentation, Friday, April 23, 2004, 3:45 – 4:10 PM*

Role of paraoxonase in organophosphate toxicity

Lucio G. Costa
University of Washington, Seattle, Washington, USA

Abstract: Paraoxonase (PON1) is a high density lipoprotein-associated enzyme involved in the metabolism of organophosphorus (OP) insecticides, nerve agents, oxidized lipids and certain pharmaceutical drugs. Single nucleotide polymorphisms have been described in the coding region (Q192R; L55M), in the 5' regulatory region (five), and in the 3' UTR (four). The Q192R polymorphism significantly affects the catalytic efficiency for metabolism of some substrates, such as the OPs, while the 5'C-108T polymorphism affects the levels of PON1. The presence of such polymorphisms has been suggested to confer differential susceptibility of individuals to the toxic effects of OPs. Verification of such hypothesis has been made possible in recent years by the use of transgenic animal models. PON1 knock-out mice were found to be extremely sensitive to the toxic effects of chlorpyrifos oxon (CPO) and diazoxon (DZO), the active metabolites of the widely used OPs chlorpyrifos and diazinon. Surprisingly, $PON1^{-/-}$ mice were not more sensitive than wild-type animals to the toxicity of paraoxon, the OP after which the enzyme was named. This can be ascribed to differences in catalytic efficiencies of PON1 toward different OP substrates. Mice were also produced that expressed human transgenes encoding either the 192R isoforms in place of mouse PON1. Both $hPON1_{192Q}$ and $hPON1_{192R}$ mice expressed similar levels of PON1, but the former were significantly more resistant to the toxicity of CPO. This is consistent with the higher catalytic efficiency of CPO hydrolysis for the $hPON1_{192R}$ isoform. These mouse models provide critical information on the role that PON1 plays in modulating the toxicity of certain OPs, and allow definition of the role of PON1 polymorphisms in susceptibility to these compounds.

Biographical Sketch:

1977	Doctoral degree in Pharmacology from the University of Milan, Milan, Italy
1978-1979	Postdoctoral Fellow, Institute of Pharmacology and Pharmacognosy, Toxicology Lab., University of Milan, Italy.
1980-1983	Research Scientist, Division of Toxicology, Department of Pharmacology, University of Texas Medical School at Houston, Texas.
1983-present	Faculty, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, Washington (Assistant Professor, 1983-1986; Associate Professor, 1987-1991; Professor, 1992-present).
1991-2000	Director, Toxicology Program, Dept of Environmental Health, UW
1985-present	Core faculty, Environmental Pathology/Toxicology Training Program, UW

DAHMANI, Yahya *Poster*

Paraoxonase 1 gene (PON1) polymorphisms and human sperm quality

Yahya Dahmani, E. Ruiz-Pesini, A. Marcuello, M.J. Lopez-Perez, C. Diez-Sanchez
Departamento de Bioqucimica y Biologica Molecular y Celular, Zaragoza, Spain

Human serum paraoxonase (PON) is an enzyme associated to high density lipoprotein (HDL) involved in lipid peroxides hydrolysis and organophosphates (OPs) degradation. The gene encoding this enzyme (PON1) shows two polymorphisms at positions 55 and 192 that define two alleles each; L and M for 55 and A and B for 192. The two last alleles show high and low lipid peroxides hydrolysis ability, respectively.

In this study we have first investigated the distribution of PON1 polymorphisms in blood of a Spanish population and in semen samples from patients coming from Hospital Reproduction Assistant Units, finding very similar genotypes distribution in both populations. We have also found that 192, but not 55, PON1 alleles are associated with sperm motility and vitality. Since A and B alleles from192 PON1 polymorphism show different capacity to hydrolyse lipid peroxides and OPs, our results allow us to propose that PON1 genetic background associate with differences in membrane lipid state and detoxifying protection of spermatozoa thus influencing male fertility.

DANTOINE, Thierry *Selected Poster Presentation, Friday, April 23, 2004, 9:40 9:45 AM*

Application of the integrated Michaelis equation to the study of paraoxonase.

Thierry F. Dantoine, Louis Merle, Jean-Pierre Charmes, Jean Debord
Dupuytren University Hospital; Pharmacology Laboratory/Department of Gerontology, Limoges, France

Paraoxonase kinetics is usually studied by the initial rate method. In many circumstances this procedure cannot be applied. It is the case, for instance, when a slow-binding inhibitor is studied, or when a significant amount of substrate is hydrolysed before the reaction product can be measured. For these reasons, we have made use of the recent mathematical developments concerning the integrated Michaelis equation (Schnell & Mendoza, *J. Theor. Biol.* 187 (1997) 207; Goudar *et al, BBA* 1429 (1999) 377) to implement an algorithm for the nonlinear least squares fitting of this equation to experimental data in which the initial substrate concentration, the enzyme concentration or the reaction time is varied. This algorithm has been incorporated into our computer program WinReg, which is freely available on the Internet. The procedure was applied to kinetic studies of human paraoxonase, using either whole serum or purified enzyme. The hydrolysis of phenyl acetate was studied at 37°C by microcalorimetry and spectrophotometry. Both techniques gave similar results. In particular, the Michaelis constant at 37°C (~ 2.5 mM) was much higher than the usual value at 25°C (~ 1 mM). Due to the narrow application range of the Beer-Lambert law, the spectrophotometric method was limited to initial substrate concentrations lower than the Michaelis constant. Microcalorimetry, on the other hand, did not display such limitations. An example of microcalorimetric results is shown in fig. 1. The extension of these techniques to the study of inhibitors is being developed in our laboratory.

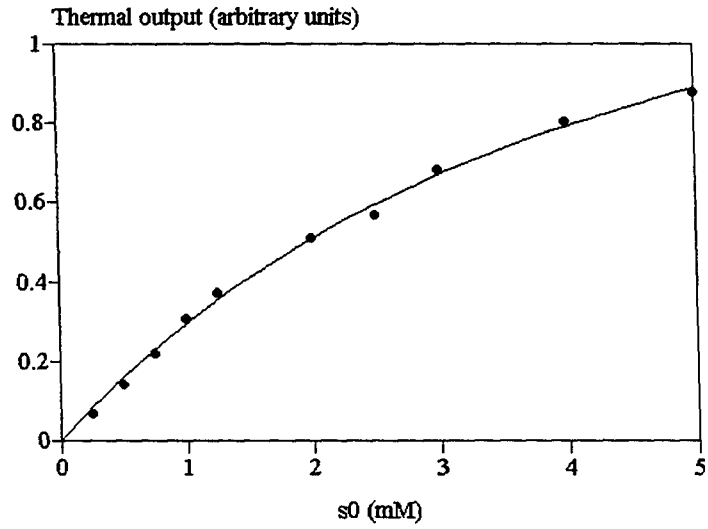

Fig. 1. Microcalorimetric study of paraoxonase: thermal ouput *vs* initial substrate concentration. The curve corresponds to the integrated Michaelis equation.

DEAKIN, Sara P. *Short Communication, Friday, April 23, 2004, 1:50 – 2:05 PM*

VLDL: An Alternative Vector for Paraoxonase 1 Secretion

Sara P. Deakin, Xenia Moren, Richard W. James
Geneva University Hospital; Department of Diabetology, Endocrinology and Nutrition, Geneva, Switzerland

Variations in serum PON1 concentrations may be important in determining susceptibility to atherosclerosis. We are investigating mechanisms of secretion of PON1 which can influence serum levels of the enzyme.

We previously proposed a mechanism by which PON1 is inserted into the external membrane of the cell and requires an acceptor complex to remove it. We showed that HDL was able to act as an acceptor complex and stabilise enzyme activity while LDL was not. This is consistent with the observation the PON1 is associated with HDL and not LDL in serum.
There are strong metabolic ties between VLDL and HDL which include exchange/transfer of apolipoproteins (C,E, AI) and lipids. In this study we examined whether VLDL was also able to act as an acceptor for PON1 secretion.

VLDL were shown to promote secretion of PON1 from a CHO model and from hepatocyes in a high affinity, saturable manner. FPLC analysis showed that PON1 is able to associate with VLDL. When HDL was presented to VLDL associated PON1 the majority of the enzyme was rapidly transferred to HDL in a process which did not require lipolysis. However a small amount of PON1 remained associated with VLDL. Low levels of PON1 were found to be associated with VLDL in human serum, and VLDL- associated enzyme activity was proportional to serum triglyceride concentrations. Although serum triglycerides were positively associated with whole serum PON1 mass they were negatively associated with specific activity. PON1-enriched VLDL was more resistant to oxidation in vitro.
This study suggests that VLDL is able to influence PON1 secretion and modulate its metabolism and activity. Whether the overall effects of VLDL on PON1 are detrimental or beneficial may depend on ambient physiological conditions. The consequences could be of relevance as the major part of any 24h period is spent in the post-prandial phase, exaggerated postprandial triglyceridaemia is a risk factor for vascular disease and diabetes is associated with a sustained increase in VLDL.

DRAGANOV, Dragomir *Invited Presentation, Friday, April 23, 2004, 9:15 – 9:40 AM*

Enzymatic Activities and Substrate Specificity for PONs 1, 2 and 3

Dragomir Draganov, John F. Teiber, Bert N. La Du
University of Michigan, Ann Arbor, Michigan, USA

Abstract: Early research on serum paraoxonase/arylesterase (PON1) focused on its ability to hydrolyze toxic organophosphates, such as paraoxon from where its name derives, and aromatic esters of acetic acid. Recently, we have demonstrated PON1's ability to hydrolyze a wide range of aromatic and aliphatic lactones and to catalyze the reverse reaction, lactonization of hydroxycarboxylic acids. PON2 does not hydrolyze organophosphates and possesses only limited arylesterase activity. PON3 has very low organophosphatase activity and different preferences for aromatic esters than PON1. However, all three PONs hydrolyze certain lactones and lactonize the corresponding hydroxyacids. Both PON2 and PON3 have more stringent substrate specificity compared to PON1. Similar to PON1, PONs 2 and 3 also require Ca^{2+} for their stability and enzymatic activity, which could be stimulated by phospholipids. All three PONs have been shown to have antioxidant properties and to detoxify biologically active oxidized lipids, but their actual substrates and the mechanism(s) of their catalytic activities remain to be elucidated. The peroxidase and phospholipase-A2 activities claimed for PON1 are critically reviewed.

Biographical Sketch: Dr. Dragomir Draganov obtained his MD degree from the Bulgarian Medical Academy, Sofia, Bulgaria in 1988 and during the same period, he acquired a Specialty in Scientific Medical Information from the Higher Institute of Medicine, Sofia (1986). He obtained a Ph.D. in Toxicology and Pharmacology in 1994, and a Specialty in Military Toxicology (1995) from the Military Medical Academy (MMA) in Sofia, Bulgaria. From 1993 to 1997, Dr. Draganov served as Research Fellow at the MMA's Laboratory of Experimental Toxicology, where he continued to evaluate drugs and test compounds as antidotes against organophosphate intoxications. Dr. Draganov continued his post-graduate studies at the Department of Physiology at the Wayne State University, Detroit, USA. In 1998, he joined Dr. Bert N. La Du's laboratory at the Department of Anesthesiology, University of Michigan, where he pursued his studies on the purification and characterization of paraoxonases in human and other species. His research aims are to elicit the roles of the different PONs in protection of organs and tissues against oxidative damage, to identify factors affecting their expression and/or activities, and to evaluate their feasibility as drug targets. Dr. Draganov is currently a Research Investigator in the Department of Pharmacology, University of Michigan.

DRAGANOV, Dragomir *Poster*

High level expression and purification of human Paraoxonases using a baculovirus system

Dragomir I. Draganov, John F. Teiber, Audrey Speelman, Roger Sunahara, Bert N. La Du
Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Human PON1 has been purified from serum, where it is associated with high density lipoprotein. Human serum PON3 levels are very low and PON2 is not present in serum at all, which makes their recombinant production the only feasible way to obtain them. Insect cell expression systems are widely used for recombinant protein production. A baculovirus mediated expression of PON1 has been shown to generate a recombinant protein functionally similar to PON1 purified from human serum [Brushia, R.J., Forte, T.M., Oda, M.N., La Du, B.N., Bielicki, J.K. (2001) J Lipid Res. 42, 951-958]. We have generated similar baculovirus mediated expression system for all three human PONs. Large quantities of recombinant human PONs were produced by HighFive™ insect cells (30-50 mg/L), associated exclusively with the crude membrane fraction of the cells. We developed an optimized procedure for purification of the recombinant PONs to apparent homogeneity, which includes extraction of the PONs from the crude membrane fraction with a detergent (*n*-dodecyl-α-D-maltoside) and three simple chromatography steps (Microprep-DEAE, Concanavalin A-Sepharose and Superdex 200). All three recombinant PONs were glycosylated with high-mannose type sugars, sensitive to endoglycosidase H digestion. Non-denaturing polyacrilamide gel electrophoresis and Ferguson plot analysis demonstrated that native enzymatically active forms of PON1 and PON3 are dimers, but PON2 forms a trimer.

This high level expression system for human paraoxonases in insect cells can provide large amounts of highly purified proteins for biochemical, pharmacological and crystallographic experiments.

FORTE, Trudy *Invited Presentation, Saturday, April 24, 2004, 10:45 – 11:10 AM*

PON1: Its Role in Athero-protection in Mice

Trudy Forte
Lawrence Berkley National Laboratories, Berkley, California, USA

Abstract: High density lipoprotein (HDL) is the major transporter of lipids in normal mice. This lipoprotein is unique in that is also transports several enzymes that putatively confer anti-atherogenic properties including paraoxonase 1 (PON1), lecithin:cholesterol acyltransferase (LCAT) and platelet activating-factor acetylhydrolase (PAF-AH). ApoE knock out (KO) mice that are prone to precocious atherosclerosis on a chow diet are characterized by elevated plasma non-HDL cholesterol, low HDL-cholesterol, elevated plasma bioactive oxidized phospholipids and decreased PON activity. These observations suggest that low levels of PON1 activity parallel low HDL concentrations; moreover, it is likely that the accumulation of oxidized phospholipids in this model contributes to the loss of PON1 activity and the precocious onset of atherosclerotic lesions. Overexpression of human PON1 in C57Bl/6 mice was shown to confer protection against aortic lesion development in this mouse strain (Tward et al, Circulation 1006:484, 2002) implicating HDL and PON1 in athero-protection. We have developed a transgenic mouse in the FVB background that overexpresses mouse PON1 (5-fold increase in activity and mass). Overexpression of PON1 did not alter HDL cholesterol levels but prevented impairment of LCAT activity when plasma was exposed to oxidative stress. Excess PON1 inhibited lipid hydroperoxide formation on HDL strongly suggesting that high levels of PON1 protect the integrity and function of HDL thus contributing to atherosclerosis protection.

Biographical sketch:

1954-1958	Immaculata College, Immaculata, PA, A.B. in Biology
1959-1964	Univ. of Pennsylvania, Philadelphia, Ph.D. in Biology
1964-1965	Univ. of Southern California, Los Angeles, Postdoc - Biochemistry
1967-1969	Univ. of California, Berkeley, Postdoc - Biophysics
1969-1977	Research Biophysicist, Donner Laboratory, LBL, Univ. of Calif., Berkeley, CA
1977-present	Senior Scientist, Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory, University of California, Berkeley, CA

Awards/Professional Activities

1976-1998	Director, NIH Institutional Training Grant
1979-1980	NSF, Member Instrumentation Panel
1981	Member, Parent Research Review Committee, Specialized Centers for Research in Arteriosclerosis, NHLBI
1987-1991	Member, NIH-NHLBI Research Training Review Committee
1990-present	Member, Graduate Group in Comparative Biochemistry, Univ. Calif., Berkeley, CA

1991-1994	Member-at-large, AHA Executive Committee, Council on Arteriosclerosis
1992-1994	Chair, Women & Minority Leadership Committee, Council on Arteriosclerosis, AHA
1993	LBL Outstanding Performance Award
1994	AWU-DOE Distinguished Lecturer
1994-1996	Vice-Chair, Council on Arteriosclerosis, AHA
1996-1997	Chair, Council on Arteriosclerosis, AHA
1990-present	Member, Editorial Board, Arterio Thromb Vasc Biol
1993-present	Member, Advisory Board J Lipid Research
2000-2003	Chair, Advisory Board, Deuel Conference on Lipids
2000	Special Recognition Award, AHA Council on Arteriosclerosis, Thrombosis and Vasc Biol
2000	Chair and organizer, First Conference on Artherosclerosis, Thrombosis and Vasc Biol
1999-present	Editor-in-Chief, J Lipid Research
2001	First Mentoring Award, Women in Artheriosclerosis Leadership Committee, AHA
2000-2004	Member of the Executive Board, International Atherosclerosis Society

FORTUNATO, Giuliana

Selected Poster Presentation, Saturday, April 24, 2004, 2:25–2:30 PM

Paraoxonase and superoxide dismutase gene polymorphisms in Italian patients with noise-induced hearing loss

Giuliana Fortunato, Federica Zarrilli[1], Cristina Mazzaccara[1], Elio Marciano[2], Paoloc La Manna, Vincenzo Marcelli[2], and Lucia Sacchetti[1]
[1]Dipartimento di Biochimica e Biotecnologie Mediche, Universitá Federico II di Napoli and CEINGE scarl, [2]Dipartimento di Neuroscienze e Scienze del Comportamento, Universitsá Federico II di Napoli, Napoli, Italy

Cochlear epithelium damage due to noise is a major cause of permanent hearing loss. In an attempt to explain the variability among subjects regarding susceptibility to noise-induced hearing loss (NIHL), we evaluated polymorphisms of Mn-Superoxide Dismutase (SOD2) and of Paraoxonases (PON1 and PON2). In fact, the local release of free radicals can damage the cochlear sensorial epithelium and so genes involved in the regulation of reactive oxygen species, such as Mn-SOD or PON2, an antioxidant enzyme ubiquitously expressed in body tissues, can affect cochlea vulnerability to noise and predispose to hearing loss. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide. Mn-SOD, located in the mitochondria, is a homotetramer and each subunit is encoded by the SOD2 gene on chromosome 6q25. The gene spans five exons and produces a 222-amino acid protein whose first 24 amino acids represent the mitochondrial targeting sequence. There are three PON genes (PON 1–3), which map to chromosome 7. Polymorphisms have been detected at codons 192 (Gln-Arg, Q/R) and 55 (Met-Leu, M/L) in PON 1, and at codon 311 (Ser-Cys,) in PON 2. Ninety-four subjects working for Alenia factory and subjected to prolonged and intense sound stimulations, were investigated for polymorphisms of SOD2, PON1 and PON2. Using an original mutation screening procedure for the SOD 2 gene based on denaturing reverse phase high performance liquid chromatography, we identified three known polymorphisms (Ala-Val (A/V) 16, IVS3-23 T/G and IVS3-60 T/G) and two new polymorphisms (IVS1+8A/G and IVS3+107T/A) in this gene. We identified the PON 1 and PON2 genotypes using PCR amplification and digestion with restriction endonuclease. Log regression analysis of data (after adjusting for age and smoking) showed that PON 2 (SC+CC) genotypes (OR 5.01; 95% CI 1.11-22.54), and IVS3-23 T/G and IVS3-60 T/G SOD2 (OR 5.09, CI 1.27-20.47) polymorphisms were associated with NIHL. These data support our hypothesis of a genetic predisposition to oxidative stress based on different polymorphisms of SOD2 and PON2 that could play a crucial role in the development of NIHL.

FURLONG, Clement *Invited Presentation, Friday, April 23, 2004, 1:00 – 1:25 PM*

Functional Genomics of PON1

Clement Furlong
University of Washington, Seattle, Washington, USA

Abstract: Human paraoxonase (PON1) is encoded by a gene on chromosome 7, one of a three-member family of genes involved in protection against oxidative stress. PON1, the most studied member of this family of genes, was first described as an A-esterase/organophosphohydrolase. Population studies showed that plasma paraoxonase activity was polymorphically distributed in human populations. Studies in La Du's laboratory and ours led to the cloning and characterization of the cDNA that encoded PON1 and the finding that a Q192R polymorphism accounted for the substrate-dependent polymorphic properties of PON1. These groups also showed that in addition to the qualitative variation in PON1 (Q192R) there was also a large variability in PON1 plasma levels among individuals. Work in the laboratories of James, Suehiro and ours showed that polymorphisms in the 5' regulatory region of PON1 accounted for some of this variability. PON1 is also developmentally regulated, reaching plateau levels between approximately 6 and 15 months of age. Early on, it was thought that "high paraoxonase" individuals would be resistant to paraoxon(PO)/parathion exposures. Our recent research has shown that it is the catalytic efficiency of PON1 that determines resistance to OP compounds and that PON1 has little influence on paraoxon sensitivity, but a major influence on sensitivity to chlorpyrifos oxon (CPO) and diazoxon (DZO) and less on sensitivity to the respective parent compounds. PON1 levels determine sensitivity to DZO, while both PON1 levels and position 192 genotype determine sensitivity to CPO. Plotting rates of DZO vs. PO for plasma samples from different individuals breaks human populations into three distinct groups, individuals homozygous for 192Q, 192R and heterozygotes and at the same time provides PON1 activity levels for each individual. This characterization has been referred to as an individual's PON1 status. Many studies on the relationship of PON1 to disease status have been carried out that consider only PON1 genotypes. Our studies note the importance of considering PON1 status as a risk factor for disease. Low PON1 status is a risk factor for carotid artery disease. Analysis of PON1 status compared with position 192 genotyping reveals discrepants who have mutations in one of their PON1 alleles (e.g., W194X; Asp124missplice; P90L and a probable deletion mutant.

Biographical Sketch: Dr. Furlong received his BA degree in Chemistry from San Jose State College (now University) in 1963 and his Ph.D. in Biochemistry from the University of California, Davis in 1968. He joined Dr. Leon Heppel at Cornell University for two-years of postdoctoral training, where he worked on the isolation and characterization of the ligand receptors for the osmotic shock sensitive (ABC) nutrient transporters in *E. coli*. He then joined the Biochemistry department at the University of California, Riverside, where he continued studies on the ABC transporter systems and began work with mammalian cells. In 1977 he moved to the University of Washington with a joint appointment in Medical Genetics and Genetics (now Department of Genome Sciences). Research on PON1 began shortly after arriving at the University of Washington in collaboration with Dr. Arno Motulsky and later with Dr. Lucio Costa.

Improving catalytic properties of recombinant PON1 toward detoxification of organophosphate compounds by in vitro evolution.

Leonid Gaydukov, Amir Aharoni, Dan S. Tawfik
Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Although serum paraoxonases PON1s are capable of hydrolysing organophosphate (OP) insecticides and nerve agents, their application *in vivo* and *in vitro* requires significant improvement in catalytic efficiency. We describe how directed evolution of recombinant, bacterially-expressed mammalian PON1 (rePON1), led to its specialization towards OP detoxification and enhancement of its specific activity.

Eight different gene libraries of rePON1 with various mutational loads were prepared by random mutagenesis. 10^3-10^4 colonies from each library were directly screened on agar plates for hydrolysis of a fluorogenic OP substrate - 7-*O*-diethylphosphoryl-3-cyano-7-hydroxycoumarin (DEPCyC - a close homologue of the insecticide Coumaphos). About 100 improved clones were isolated, and the best variants combined by shuffling to construct a second generation library. This library was then submitted to a second round of screening resulting in the identification of PON1 variants with dramatically improved OP-hydrolyzing activity and specificity.

The newly evolved variants exhibited a 40-fold increase in catalytic proficiency (k_{cat}/K_M) towards DEPCyC compared to wild-type PON1. This was accompanied by a drastic decline (50-fold) in carboxy-esterase activity, thereby giving a specificity shift of >2000-fold and producing PONs specialized for OP rather than ester hydrolysis. A few apparently subtle amino acid substitutions were responsible for the enhanced organophosphatase activity; namely L69V, S193P, V346A (major) and V48I, N105D and N287D (minor). Interestingly, screening the mutagenic libraries with a fluorogenic carboxy-ester substrate, 7-acetoxycoumarin, also led to the isolation of PON1 variants with >10-fold improved esterase activity and a decreased OP-hydrolysing activity, confirming that PON1 provides a hitherto unique starting point for the evolution of proficient "specialized" enzymes.

This study demonstrates that PON1 is amenable to the powerful tool of directed evolution and opens new prospects for improving its phosphotriesterase activity against other OPs, as well as other catalytic activities related to atherosclerosis.

HALEY, Robert W. *Invited Presentation, Friday, April 23, 2004, 4:35 - 5:00 PM*

Neuroepidemiologic Requirements for Detecting Associations of PON1 Isoenzyme Activity with Gulf War Illness

Robert W. Haley
University of Texas Southwestern Medical Center, Dallas, Texas, USA

Abstract: Many Gulf War veterans remain ill with a condition resembling the early stage of a neurodegenerative disease typified by neuropsychiatric symptoms without objective clinical signs. Similarities to chronic symptoms in survivors of the sarin attack in the Tokyo and Mastumoto subways, widespread troop exposure to low-level sarin and organophosphate (OP) pesticides in the Gulf War, and evidence of brain cell damage from low-level sarin in animal models suggest the hypothesis of sarin-induced organic brain syndrome in individuals with genetic predisposition to OP neurotoxicity. Addressing this hypothesis in human studies, however, is complicated by extreme difficulty in formulating a case definition that separates the suspected environmentally caused condition from two-fold greater background illness where disability compensation is at issue.

Our studies began with development of a case definition by factor analysis of symptoms in 249 members of a military battalion surveyed in a group setting with a survey form developed from interviews with typically ill veterans. The analysis identified three common symptom complexes involving 63 ill veterans; 116 others claimed war-related illness but with non-clustering symptoms. Veterans with symptom complex 2 were severely disabled (mean SF-36 Physical Functioning [PF] scale, 35) and those with symptom complexes 1 and 3 were only moderately ill (PF, 72 and 60, respectively), compared with controls (PF, 92). Epidemiologically only symptom complex 2 was strongly associated with self-reported exposure to low-level nerve gas ($RR = 7.8$, $p < 0.0001$) and being up near the front line on the day sarin was detected in ambient air ($RR = 4.3$, $p = 0.004$).

Clinical studies for objective markers found symptom complex 2 with significantly reduced N-acetyl-aspartate concentrations by MR spectroscopy in both basal ganglia compared with controls; whereas, symptom complex 1 had marginally significant reductions only in basal ganglia and symptom complex 3, only in brainstem. Analysis of plasma paraoxonase and arylesterase assays demonstrated substantially lower activity of type Q isoenzyme of PON1 in symptom complex 2 (mean 56 $\pm$ SEM 10 U/ml) and intermediate levels in symptom complexes 1 (72 $\pm$ 11) and 3 (69 $\pm$ 7) compared with controls (88 $\pm$ 11; $p = 0.009$). Type R and total paraoxonase were not associated, but having an R allele was weakly associated ($RR = 3.5$, $p = 0.05$).

We suggest that a tight case definition modeled on clustering of typical symptom descriptions avoids misclassification in the illness measure that would bias studies strongly toward the null hypothesis and is necessary to demonstrate associations with objective markers.

Biographical Sketch: Robert W. Haley, M.D., is professor of internal medicine and director of the Division of Epidemiology in the Internal Medicine Department at the University of

Texas (UT) Southwestern Medical Center at Dallas and holder of the U.S. Armed Forces Veterans Distinguished Chair for Medical Research Honoring America's Gulf War Veterans. He received his M.D. degree from UT Southwestern Medical School and served a residency in internal medicine at Dallas Parkland Memorial Hospital. He spent 10 years (1973-1983) at the U.S. Centers for Disease Control and Prevention (CDC), where he investigated epidemics, performed research in nationwide samples of U.S. hospitals, and integrated laboratory and epidemiologic units into a unified Hospital Infections Program. In 1983, he founded the Division of Epidemiology and Preventive Medicine at UT Southwestern. He is an attending physician at Parkland Memorial Hospital and has conducted research on the epidemiology and prevention of hospital-acquired infections, hepatitic C, and Gulf War syndrome. Beginning in 1994 his research on ill Gulf War veterans was the first to establish a case definition of the Gulf War syndrome with which to study brain cell metabolic abnormalities by brain imaging and neurophysiologic testing. In 1997 he undertook studies of paraoxonase isoenzymes in collaboration with Dr. Bert La Du to test for a genetic predisposition to chronic neurotoxic injury from organophosphate nerve agent in ill Gulf War veterans.

HALEY, Robert W. *Poster*

Low Type Q Paraoxonase/Arylesterase in Ill Gulf War Veterans Not Due to Circulating Proinflammatory Cytokines

Robert W. Haley, Scott Billecke, Bert N. La Du
University of Texas Southwestern Medical Center; Department of Internal Medicine, Dallas, Texas, University of Michigan, Ann Arbor, Michigan, USA

Recently Hotopf et al. (*J Occup Environ Med* 2003;45:668-675) demonstrated that low plasma concentrations of total paraoxonase activity in ill British Gulf War veterans was not due to suppression by increased intracellular cytokines, but circulating cytokines were not measured. To addresses the latter, we analyzed plasma concentrations of proinflammatory cytokines measured simultaneously with the paraoxonase allozyme activity measurements reported from our previous study of Gulf War veterans.

In the subset of 39 subjects (22 cases with 3 Gulf War-related symptom complexes and 17 controls) who were hospitalized for 7 days in the UT Southwestern Medical Center's General Clinical Research Center, venous blood was uniformly drawn at 7:30 a.m. on day 6 of the protocol and analyzed for paraoxonase allozymes at University of Michigan and for proinflammatory cytokines IL-1β, IL-6 and TNF-α at Mayo Medical Laboratories.

Elevated concentrations were found for TNF-α in 4 subjects (3 cases and 1 control; $p = 0.6$), for IL-1β in 4 subjects (2 cases and 2 controls; $p = 1.0$), and for IL-6 in 4 subjects (1 case and 3 controls; $p = 0.3$). One subject had elevated levels of two cytokines (a control with elevated TNF-α and IL-6). Eleven subjects had at least one elevated cytokine (6 cases and 5 controls; $p = 1.0$).

In logistic regression analysis of the 12 cases with symptom complex 2 (confusion-ataxia) and 17 controls, the lowest quartile of type Q paraoxonase/arylesterase allozyme (PON-Q) was significantly associated with being a case (odds ratio, 14.0; 95% CI 2.3-85.2; $p = 0.004$). After controlling for having any elevated proinflammatory cytokine, the association remained significant (odds ratio, 15.7; 95% CI 2.3-105.7; $p = 0.005$).

In the 22 cases with any of the three symptom complexes and 17 controls, the lowest quartile of PON-Q was significantly associated with being a case (odds ratio, 6.7; 95% CI 1.5-30.5; $p = 0.013$). After controlling for having any elevated proinflammatory cytokine, the association remained significant (odds ratio, 7.6; 95% CI 1. 6-36.9; $p = 0.012$).

In the U.S. Gulf War veterans studied, inflammatory states reflected by elevated circulating proinflammatory cytokins occurred approximately equally in cases and controls and did not account for the association between low PON-Q allozyme activity and Gulf War-related symptom complexes.

van HIMBERGEN, Thomas

Short Communication, Saturday, April 24, 2004, 9:35 – 9:50 AM

Paraoxonase contributes to plasma high-density lipoprotein levels in patients with familial hypercholesterolemia

Thomas M. van Himbergen[1,2], MSc; Mark Roest[1], PhD; Jacqueline de Graaf[2], MD, PhD; Eugène H.J.M. Jansen[3], PhD; Hiroaki Hattori[4], PhD; John J.P. Kastelein[5], MD, PhD; Hieronymus A.M. Voorbij[1], MD, PhD; Lambertus J.H van Tits[2], PhD; Anton F.H. Stalenhoef[2], MD, PhD

[1]Research laboratory of the department of Clinical Chemistry, UMC Utrecht, Utrecht, [2]Department of Medicine, Division of General Internal Medicine, UMC Nijmegen, Nijmegen, [3]Laboratory for Health Effects Research, National Institute for Public Health and Environment, Bilthoven, [4]Department of Advanced Medical Technology and Development BML, Inc., Saitama, Japan, [5]Department of Vascular Medicine, Academic Medical Centre of Amsterdam, Amsterdam, The Netherlands

Background. Serum paraoxonase (PON-1) is a high-density lipoprotein (HDL)-associated enzyme, that mainly protects low-density lipoprotein (LDL) against oxidative modification. It also inhibits the oxidation of HDL, thus preserving HDL function. Both of these properties may contribute to the protection against cardiovascular disease (CVD). We assessed the consequences of PON-1 levels, activity and molecular variation at the PON-1 gene locus in terms of their effect on HDL-cholesterol levels and on the carotid intima media thickness (IMT), a surrogate marker of CVD.

Method. PON-1 genotypes (L55M, Q192R, -107C/T, -162A/G, -824G/A, and -907G/C) were determined in 302 patients with familial hypercholesterolemia (FH). PON-1 levels were measured using PON-1-specific antibodies. PON-1 activity was monitored by the hydrolysis rate of paraoxon, diazoxon, and phenyl acetate.

Results. The genetic variants of PON-1 that were associated with high levels and activity of the enzyme were associated with higher HDL-cholesterol levels (p values for trend: 0.008, 0.020, 0.042, and 0.037 for L55M, Q192R, -107C/T, and –907G/C, respectively). There was a positive correlation between PON-1 levels and activity and HDL-cholesterol (PON-1 levels: $r=0.37$, $p<0.001$; paraoxonase activity: $r=0.23$, $p=0.01$; diazoxonase activity: $r=0.29$, $p<0.001$; arylesterase activity: $r=0.19$, $p=0.03$). Neither the HDL-cholesterol levels nor the PON-1 genotype, levels, and activity were associated with carotid IMT.

Conclusions. Our observations support the hypothesis that PON-1 preserves HDL-cholesterol levels in plasma. The relevance of this supposedly anti-atherogenic activity needs to be delineated in further studies with clinical endpoints of CVD.

This study was financially supported by Research Grant #2001.038 of the Netherlands Heart Foundation.

JAKUBOWSKI, Hieronim

Short Communication, Saturday, April 24, 2004, 1:40 – 1:55 PM

Determinants of homocysteine-thiolactonase activity in humans

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Homocysteine (Hcy)-thiolactonase/paraoxonase (HTase/PON1) detoxifies Hcy-thiolactone in human blood and could thus delay the development of atherosclerosis [Jakubowski H. J Biol Chem 2000;275: 3957-62; Jakubowski et al. FEBS Lett 2001;491:35-9]. To gain insight into physiological role(s) of the PON1 protein, we studied HTase and PON1 activities, as well as *PON1* genotypes in 98 subjects with angiographically proven coronary artery disease (CAD), 73 of whom suffered myocardial infarction (MI), and in age-matched 60 healthy controls (mean age 57.5 years). We found that HTase and PON1 activities were strongly correlated ($r = 0.68$-0.93, $p<10^{-6}$, irrespective of *PON1-192* and *-55* genotypes), which suggests that the artificial PON1 activity can be used as a surrogate for the natural HTase activity. HTase activity was negatively correlated with age ($\beta=-0.23$, $p=0.003$), plasma total Hcy (in *192-QR* subjects only; $r=-0.42$, $p=0.001$). HTase activity was positively correlated with total cholesterol ($\beta=0.30$, $p<0.001$), but not with HDL cholesterol. HTase activity was similar in CAD subjects and in controls (*192QQ* genotype: 6.5±1.6 units *vs.* 6.7±2.3 units, p=0.661; *192QR* genotype: 12.7±3.7 units *vs.* 11.9±3.0 units, p=0.374; *192RR* genotype: 17.5±2.9 units *vs.* 19.9±3.6 units, p =0.187; all genotypes mediane 8.0 units (range 2.2–21.2) *vs.* 8.3 (range 2.9–25.8), p=0.86). There were no differences in PON1 activity between CAD subjects and controls (*192*-QQ: 84.6±47.0 units *vs.* 81.0±28.2 units, p=0.657; *192*-QR: 245.9±82.5 units *vs.* 213.2±68.8 units, p=0.145; *192*-RR: 347.9±72.0 units *vs.* 438.2±89.0 units, p=0.057; all genotypes mediane 113.3 units (range 44.0–498.0) *vs.* 106.0 units (range 40–560), p=0.68). *PON1* genotypes were similarly distributed in subjects and controls (*PON1-192*: QQ, 54.1% vs. 56.7%; QR, 37.8% vs. 33.3%; RR, 8.2% *vs.* 10.0%; *PON1-55*: LL, 42.9% *vs.* 38.3%; LM, 41.8% *vs.* 51.7%; MM, 15.3% *vs.* 10.0%). These results suggest that PON1 genotype, total Hcy, total cholesterol, and age are major determinants of HTase activity in humans. However, HTase activity appears not to be associated with CAD or MI in our study population.

JAMES, Richard W. *Invited Presentation, Friday, April 23, 2004, 1:25 – 1:50 PM*

The Impact of Genetic and Non-Genetic Factors on Serum Paraoxonase Activity

Richard W. James
University Hospital, Geneva University, Geneva, Switzerland

Abstract: The serum enzyme, paraoxonase, has a number of disparate activities that can have important consequences for human physiology. It is implicated in drug metabolism and the metabolism of several organophosphate derivatives. More recently, a role in vascular disease was proposed, based on human and animal studies showing that paraoxonase offers a measure of protection against atherosclerosis. It has been linked to an anti-oxidant function of the enzyme, and its ability to limit or prevent peroxidation of serum lipids. The transport vector for paraoxonase is high density lipoproteins (HDL) with which the enzyme is entirely complexed in human serum. HDL have a powerful, negative correlation with the risk of vascular disease; one facet of the cardio-protective role of HDL is their anti-oxidant / anti-inflammatory function. Paraoxonase appears to be a major determinant of the anti-oxidant capacity of HDL. There are wide variations in serum levels of paraoxonase, which will influence the efficiency of the enzyme in the different physiological roles outlined above. The latter consideration has been a driving force behind one of our research interests, which is factors that can influence serum activity of the enzyme. We identified polymorphisms of the paraoxonase gene promoter with a powerful impact on gene expression. These have been linked to transcription factors and their influence on promoter activity. Defining the role of transcription factors has allowed us to identify potential pathways involved in gene expression. Our studies have also highlighted the impact of non-genetic factors on serum paraoxonase. These include age, diabetes and smoking and may reflect the underlying susceptibility of paraoxonase to the redox state. Finally, studies that examined mechanisms by which paraoxonase is released from the liver revealed another pathway which could influence serum levels of the enzyme, as well as emphasising the importance of HDL for secretion and stabilisation of the enzyme.

Biographical Sketch: Dr. James undertook his undergraduate studies at the University of Bath, UK, where he obtained an honours degree in Applied Biochemistry. He pursued his research studies with a joint appointment at the University of Bath, UK and the Agrochemicals Division of Shell Ltd, Kent, UK, looking at membrane proteins involved in signal transmission at the synaptic junction. This led to a PhD in Biochemistry (1976). Dr. James continued his interest in the biochemistry and biology of membrane proteins with an EMBO postdoctoral fellowship at the Biochemistry Department, University of Geneva, Switzerland. Here he developed an interest in the implications of membrane proteins in disease, with studies of the acetylcholine receptor. A growing interest in medically-oriented research and collaborations with groups from the medical school lead to an appointment in the Lipid Laboratory, Division of Diabetes, University Hospital, Geneva (1981) where he exploited his knowledge of the biochemistry of hydrophobic proteins to study apolipoprotein components of lipoproteins. His principal research activities are the metabolism of lipoproteins in diabetes and insulin resistant states, with particular focus on high density

lipoproteins. Studies in the early 1990's, where his group identified and isolated a high density lipoprotein sub-class defined by paraoxonase, lead to his present interest in the enzyme, and its anti-oxidant role. Dr. James is currently head of research in the Lipid Laboratory, Division of Endocrinology and Diabetes, University Hospital, Geneva with a joint appointment as Clinical Lecturer in the Medical Faculty, University of Geneva, Switzerland.

PON1 and the Antiatherogenic Properties of HDL: Effect of Aging

Leila Jaouad, Chantal de Guise, Chantal Bélanger, Christelle Milochevitch and Abdel Khalil
Research Center on Aging, Department of Medicine, University of Sherbrooke, Québec, Canada

Paraoxonase (PON1) is principally complexed to HDL and is responsible, at least in part, for its antioxidant properties. PON1 activity decreases in several pathologies associated with atherosclerosis. Our group has previously demonstrated that HDL oxidation is accompanied by a reduction in the PON1 paraoxonase activity. In parallel, paraoxonase activity showed a negative correlation with age. Therefore the objective of this study was to investigate the influence of the decreased paraoxonase activity with aging on the antiatherogenic proprieties of HDL.

Our results show a significant decrease in the antioxidant activity of HDL obtained from elderly subjects, as assessed by the conjugated diene, lipid peroxide and MDA formation: % of protection = 30 % for HDL from elderly subjects *v.s.* 64 % for HDL from young subjects. In addition, PON1 purified from plasma of elderly subjects showed a lower antioxidant activity when compared to PON1 purified from young subjects, 47.08% vs. 78.14% respectively ($p<0.001$). Moreover direct oxidation of the purified PON1 in the presence of $^{\cdot}OH/O2^{\cdot-}$ free radicals induces a significant reduction in the paraoxonase enzymatic and antioxidant activities accompanied by a decrease in the number of free sulfhydryl groups on PON1. Similarly we noted a reduction in the number of free sulfhydryls in the PON 1 purified from elderly subjects.

The present study demonstrates, for the first time, that HDL loss their antioxidant properties with aging, which may be due to the decrease of the paraoxonase activity. In conclusion, compositional alterations in HDL from elderly subjects and the loss of their antioxidant activity may accelerate the atherosclerosis process with aging.

KOCKAR, Feray *Poster*

Study of serum paraoxonase activity from brain tumour patients in Turkey

Feray Kockar[1], Selma Sinan[1]; Hatice Bozkurt[1]; Nahit Gencer[2]; Arslan Oktay[2]
[1]Faculty of Science and Literature, [2]Department of Biology, Balikesir University, Balikesir, Turkey

Paraoxonase (PON1) (EC 3.1.1.2) is a calcium dependent serum enzyme belonging to the class of A-esterases and closely associated with high-density lipoprotein complex. Two common polymorphic sites that is one at amino acid 55 and another at residue 192 have been reported and these polymorphisms are being studied for its allelic association with a number of diseases such as cardiovascular disease, carotid atherosclerosis, Parkinson and Alzheimer's disease. Some research indicates that there is a possible association of PON1 polymorphism in the pathophysiology of a neurodegenerative disorders such as Alzheimer disease. Some important physiological role of PON1 enzyme was also reported in several tissues including brain from some expression studies.

In this study, with the light of evidence of the possible role of PON1 in brain tissue, we have investigated whether if there is any association PON1 polymorphism and development of the brain tumour. Therefore, serum paraxoanase activity and the phenotype distribution of two different polymorphism types of the paraoxonase (PON 1) gene leading to a methionine (M allele)- leucin (L allele) interchange at position 55 and on arginine (B allele)-glutamine (A allele) interchange at position 192 are from Turkish brain tumour patients. We have included 30 brain tumoured patients in a various stages and 100 healty subjects as control group.

The serum paraoxonase activity in this group was determined and the comparison of serum paraoxonase activity between tumoured patients and healthy subjects was performed and the possible PON1 polymorphisms sites were also found. Furthermore the possible role of paraoxonase activity on the tumour development was further discussed.

LA DU, Bert N. *Invited Presentation, Friday, April 23, 2004, 8:50 – 9:15 AM*

Evolution and Phylogenetic Relationships of the Paraoxonases

Bert N. La Du, Dragomir Draganov
University of Michigan, Ann Arbor, Michigan, USA

The paraoxonase family of enzymes can be traced back to a lactonase of the fungus, *Fusarium oxysporum*, which has appreciable structural homology with human serum PON1 and they share several substrates, such as homogentisic acid lactone and dihydroxycoumarin. Mammals have three PONs (PON1, PON2 and PON3) named in order of their discovery. They show marked structural homology and are assumed to have arisen by gene duplication, with PON2 the oldest, followed by PONs 3 and 1. Only PON1 has paraoxonase activity, but some arylesterase activity and lactonase activities are shared by all three PONs. Another relative of the PON family is the mammalian DFPase which is now known to be identical with the senescence marker protein-30 (SMP-30), which can also hydrolyze soman and sarin, but not paraoxon. Several PONs are present in *C elegans*, at least one of which is involved in the perception of gentle touch. The variety of functions mentioned in this brief summary for the mammalian PONs is further complicated by the special ability of the PONs to protect against oxidative damage in model oxidative systems using oxidized LDL lipids or phospholipids. Most of the serum PON1 is carried in the HDL fraction, and this fraction protects against oxidative damage, but not so for HDL fractions from PON1 "knockout" mice. Since the latter develop atherosclerosis when placed on an atherogenic diet, it is suspected that the PONs may serve in some protective role to prevent the vascular and cellular pathology in several other conditions, such as diabetes. Studying the redundancy of special functions and enzymatic activities of different members of the PON family may be a useful approach to discovering their physiological roles.

Biographical Sketch: Dr. La Du obtained his B.S. degree from Michigan State College (now University) with a major in Chemistry, and an M.D. degree from the University of Michigan Medical School and then a Ph.D. degree in Biochemistry from the University of California (Berkeley). He joined the Bernard B. Brodie lab of Chemical Pharmacology at the National Heart Institute and worked on drug metabolism, and wrote one of the first two papers on the liver microsomal enzyme system (dealkylation of N-alkylamines) that later became known as one of the P-450 systems. He then moved to the National Institute of Arthritis and Metabolic Diseases and identified the enzymatic deficiency in two metabolic disorders of amino acid metabolism: alkaptonuria and histidinemia. He spent a year on sabbatical in human genetics at the Galton Laboratory in London and then moved to New York University to become Chairman of the Pharmacology Department. There he started his studies on pseudocholinesterase characterizing the kinetic properties of purified variant forms of the enzyme in patients with drug sensitivity to the muscle relaxant succinylcholine. In 1974 he moved back to Ann Arbor as Chairman of Pharmacology and continued the research on cholinesterase. His laboratory determined the complete sequence of the enzyme, cloned the gene and developed methods to characterize variants at the DNA level, diagnose complicated

mutations and do family studies to follow the patterns of these allelic variations. Over 35 distinct variants were identified as specific mutations affecting the structure of the coding region of this enzyme. Dr. La Du's laboratory has become the international center for research on this topic.

Over the last 20 years Dr. La Du has also focused on studying serum paraoxonase (PON1). His laboratory sequenced most of the enzyme, cloned it to identify mutations and polymorphic sites, and found that paraoxonase is one member of a gene family, which includes also PON2 and PON3. Only PON1 has paraoxonase activity; the others do not hydrolyze organophosphate compounds. However, all PONs do share lactonase activity, which was also discovered in his laboratory, and lactone substrates include several statin drugs and spironolactone. Current studies are looking for the physiological functions of the PONs, their ability to prevent oxidative damage of cells and tissues and what substrates and reactions are involved in their protective activities.

The difference of human and pig serum arylesterase in the hydrolysis of different para-substituted phenyl acetate substrates

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[1]Biochemistry Department, Chongqing University of Medical Sciences, [2]Biochemistry Department, Chongqing University of Post and Telecommunication, Chongqing, P. R. China.

Arylesterase (Paraoxonase) in vertebrate serum is characterized by its calcium-dependent hydrolytic action on aromatic esters and organophosphates. But its action mechanism and physiological substrate remain unclear. For kinetically homogenous arylesterase from human and pig serum, the Michaelis-Menten constant (K_m) and relative maximum reaction rate (V_m) on different *para*-substituted phenyl acetate substrates were compared. K_m was determined by Lineweaver-Burk plot, and V_m was determined by nonlinear fitting to the reaction curve with an integrated rate equation, both with CV <10%, respectively. Their K_m showed no difference for either substrate while their V_m on these substrates exhibited great differences (Table 1). These substrates differed only by the substitutes at the *papa*-position. Their K_m profile suggested dipole interaction between residual in activei site of enzyme and the substitute at the *para*-position might be involved in substrate binding. Their profile of catalytic capacity suggested that nucleophilic attack on the carbonyl carbon might be rate-limiting with pig serum arylesterase, while the release of product from the active site might be rate-limiting with human serum arylesterase. Their differences in substrate specificity indexed by V_m/K_m may result from the process of biochemical adaptation to the life style during evolution based on the putative detoxification role of arylesterase in serum.

Table 1 Kinetic parameters of human and pig serum arylesterase on different substrates

parameters	species	4-nitrophenyl acetate	4-chlorophenyl acetate	4-methoxyphenyl acetate	phenyl acetate
K_m (mmol/L)	Human	0.24	0.25	0.84	0.96
	pig	0.25	0.26	0.86	0.94
V_m (ratio)	Human	0.016	0.018	<0.005	1.000
	pig	0.310	1.000	<0.015	0.040

MACKNESS, Bharti *Invited Presentation, Friday, April 23, 2004, 4:10 – 4:35 PM*

New Toxicological Aspects of Paraoxonase

Bharti Mackness
University of Manchester, Manchester, UK:

Abstract: Human serum paraoxonase (PON1) detoxifies organophosphate (OP) insecticides and nerve gases. There has been much debate as to whether OP's have chronic effects on health, as well as their acute effects. In a series of clinical epidemiological studies of sheep farmers, Turkish farmers using OP's to attempt suicide, Spanish glass-house sprayers and Gulf War service personnel, we have shown that the PON1 isoforms are intimately involved in determining the chronic and acute toxicity of OP's.

Biographical Sketch:

1979 B.Sc. (2.ii) Biological Sciences, Aston University
1980 M.Sc. Applied Genetics, University of Birmingham
1984 Ph.D Wolverhampton Polytechnic

1991-1994 Guide Dogs for the Blind Association Fellow, Manchester University
Sept 1995-July 1998 Research Fellow, Department of Medicine, University of Manchester (funded by the MRC
Aug 1998-Dec 1999 Research Fellow, (funded by the Lipid Research fund)
Jan 2000-Dec 2002 Research Fellow (funded by the BHF)"Effect of Paraoxonase Genetic Polymorphisms on Substrate Specificity
Jan 2002-Dec 2003. International HDL Research award (Pfizer)

MACKNESS, Michael *Invited Presentation, Saturday, April 24, 2004, 8:30 – 8:55 AM*

Paraoxonase and Atherosclerosis: Is the Gene or the Protein More Important?

Michael Mackness
University of Manchester, Manchester, UK

Abstract: PON1 has been proven in vitro and in vivo to be an anti- inflammatory/ anti-atherosclerotic component of HDL. PON1 levels are determined by a combination of complex genetic interactions and environmental/dietary factors giving rise to a 40 fold interindividual variation in activity. Despite this, most clinical epidemiological studies have concentrated on the genetics of PON1 in relation to CHD. This paper will argue that this approach is illogical and that the properties of the enzyme itself are much more important in determining its role in CHD development.

Biographical Sketch:

1975-1978 Portsmouth Polytechnic Upper Second Class B.Sc. (Hons.) Biological Sciences (C.N.A.A.)

1978-1981 PhD Department of Physiology and Biochemistry, University of Reading

1982-1984 National Kidney Research Fund Fellow, Department of Biological Sciences, Wolverhampton Polytechnic

1984-1986 Wellcome Trust Postdoctoral Fellow, Department of Physiology and Biochemistry, University of Reading.

1985 Royal Society Travelling Fellow, University of Mainz, Federal Republic of Germany.

1986-1988 MRC Fellowship, University of Reading right1988-1995 Research Biochemist, Department of Medicine, University of Manchester.

1989 FEBS Travelling Fellow, University of Kiel, Federal Republic of Germany. Visiting Fellow, University of Michigan.

Current Position Non-clinical Lecturer, Department of Medicine, University of Manchester

MANRAJ, Meera *Selected Poster Presentation, Saturday, April 24, 2004, 2:20 – 2:25 PM*

Population-based and family studies suggest an association between PON1 Gln192Arg polymorphism and premature coronary heart disease in female Mauritians of Indian origin.

Meera Manraj, Stephan Francke, HC)bC), Annick; Corset, Laetitia; Lee Kwai Yan, Solange; Sem Fa, Lan Heng; Lecoeur, CC)cile; Ramjutun, Uday, S; Gunness, Teman, K; Froguel, Philippe. University of Mauritius; SSR Centre for Medical Studies & Research, Moka; Mauritius

Serum paraoxonase is an HDL-bound enzyme protecting LDL-cholesterol from oxidation. Its encoding gene PON1 is considered to be a potential candidate gene involved in the pathophysiology of atherosclerosis. We studied the association between PON1 *Gln192Arg* polymorphism and coronary heart disease (CHD) in an Indo-Mauritian population through case-control and family based association studies.

Methods: We used PCR-RFLP techniques to study the PON1 *Gln192Arg* polymorphism in 290 male and 50 female consecutive patients with premature CHD (onset of CHD below 60) who were compared to 125 male and 56 healthy female controls. We also studied for this polymorphism 155 nuclear families (541 individuals), ascertained through an index subject with premature CHD.

Results: Allele frequency of the common variant *Gln192* allele was higher in CHD female patients compared to female control individuals (64% v/s 49%, p=0.03) but was the same in male CHD patients and control individuals (57% v/s 56%, p=0.84). We found an excess of homozygous individuals for the *Gln192* allele (44% v/s 20%, p=0.025) only in female CHD patients. When we grouped individuals with *Arg/Arg* and *Gln/Arg* genotypes and compared them to those with the homozygous *Gln/Gln* genotype, we found a significant interaction between gender and the *Gln/Gln* genotype on occurrence of CHD phenotype (p=0.02). We also found through family studies, in females only, a trend towards preferential transmission of the *Gln192* allele to female siblings affected by CHD (n=65) compared to transmission of the same allele to unaffected female siblings (n=182), p=0.04.

Conclusions: An interaction was shown between gender and the PON1 *Gln192Arg* polymorphism on the CHD phenotype in our case-control studies while family based studies confirmed both association and linkage between the polymorphism and premature CHD in Indo-Mauritian women.

MONROY-NOYOLA, Antonio *Poster*

Reduction of Serum Paraoxonase Activities in Mexican Stroke Patients

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The human serum HDL-associated paraoxonase (PON1) has two common genetic polymorphisms; one at positions 55, and another at position 192. These PON1 polymorphisms are believed to be important in determining the capacity of HDL to protect LDL from oxidative modification, and, in this way, account for the relationship between certain PON1 alleles and atherosclerosis as well as cerebrovascular disease, such as stroke. The main objective of this work was to study the serum PON1 activities and PON1 polymorphic frequencies in a sample Mexican population with stroke (n=33) selected from the National Institute of Neurology and Surgery of Mexico, and healthy aged matched controls (n=37). All the subjects were genotyped for the two polymorphic sites by standard DNA analyses and phenotyped by measuring both serum paraoxonase (paraoxon) and arylesterase (phenyl acetate) activities. For the 192 Q/R polymorphism, the respective allelic frequencies were 0.42/0.58 for the control group, and 0.47/0.53 for the stroke group. For the position 55 L/M polymorphism, the respective allelic frequencies were 0.82/0.17 for the control group and 0.80/0.20 for the stroke group. Although there are no appreciable differences in the allelic frequencies and distributions between the stroke and control samples, there was a significant reduction in both the arylesterase and paraoxonase levels of activity in the two groups. The average arylesterase activities in the control and stroke groups were 93.2±18.2 and 68.1±16.4 units/ml, respectively ($p < 0.001$), and the average paraoxonase activities were 493.8±210.5 and 333.6±167.8 units/ml, respectively ($p = 0.008$). Whether the lower values in the stroke patients existed before the stroke events or followed them, or resulted from the subsequent therapy, diet and medication is not known at this time.

Acknowledgement. We gratefully acknowledge the Fundacion Miguel Aleman, A.C. (Mexico) for supporting this research.

The relationship between PON1 Q192R genotype and phenotype in human liver

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PON1 is an esterase which is synthesised in the liver and secreted into the blood serum. It hydrolyses the active metabolites (oxons) of organophosphate pesticides (OPs) such as parathion, chlorpyrifos and diazinon. Human PON1 has a polymorphism in the coding region (Q192R) which results in two isoforms, Q (Glu) and R (Arg), which differ in their hydrolytic activity towards OP oxons.

This study aimed i) to define the rates of paraoxon, chlorpyrifos-oxon and diazoxon hydrolysis by a panel of human liver microsomes (n=27 or n=19, diazoxon hydrolysis) ii) to determine the relationship between hydrolysis of the oxons and $PON1_{192}$ genotype.

Genomic DNA was extracted from the livers using phenol/chloroform and single strand conformational polymorphism (SSCP) analysis of the PCR product was used to separate the three genotypes.

As with serum, individuals with the RR genotype preferentially hydrolysed paraoxon in liver, while chlorpyrifos-oxon and diazoxon were hydrolysed within the three genotypes at similar rates. This study showed wide rates of hydrolysis of the three oxons between individuals with the same $PON1_{192}$ genotype, indicating that functional activity may be of more importance than genotype alone.

Following an occupational dermal exposure to the parent OP, the OP would be activated by the P450s during first-pass metabolism in the liver. The small amount of oxon formed would be rapidly hydrolysed by hepatic PON1 (and other esterases) and therefore the individual's functional expression of PON1 *and* P450 may be a more relevant determinant of susceptibility to OP toxicity than PON1 genotype alone.

Table 1 Oxon hydrolysis by human liver microsomes expressed as nmol/min/mg protein.

PON1 Q192R Genotype	Paraoxon (1mM) hydrolysis	Chlorpyrifos-oxon (500μM) hydrolysis	Diazoxon (500μM) hydrolysis
All	0.170-9.60 (1.43)	17.9-288.1 (95.9)	42.7-243.6 (76.1)
QQ	0.173-4.42 (1.04)	17.9-240.1 (95.9)	42.7-176.1 (88.2)
QR	0.680-9.60 (1.20)	34.4-288.1 (44.4)	47.4-185.6 (114.3)
RR	2.53-5.96 (3.82)	39.0-221.7 (134.8)	49.5-243.6 (58.1)

NAVAB, Mohamad *Invited Presentation, Saturday, April 24, 2004, 11:35 – 12:00 AM*

ApoA-I mimetic peptide forms HDL like particles containing paraoxonase 1 in apo E null mice

Chris Chyu, Nicholas Yu, Susan Hama, Greg Hough, G. M. Anantharamayah and Mohamad Navab.

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Abstract: Orally administered apolipoprotein A-I mimetic peptides with class A amphipathic helices have been shown to markedly reduce atherosclerotic lesions in LDL receptor deficient and in apo E null mouse. They have additionally been demonstrated to posses powerful antiviral activity against influenza\a infection in mice. We have investigated the mechanism of action of an 18 amino acid apoA-I mimetic peptide in preventing the progression of atherosclerosis in theses mouse models. Following an oral dose of the apo A-I mimetic peptide, plasma was obtained from apo E null mice and particles that eluted following HDL during FPLC fractionation were studied. These cholesterol containing particles contained a significant PON 1 activity while the fractions containing mature HDL showed reduced PON 1 activity following apoA-I mimetic peptide dose compared to controls. The HDL like particles were capable of preventing LDL oxidation and the resulting monocyte chemotactic activity. These results suggest that following intestinal absorption, the apo A-I mimetic peptide accepts PON 1 from remodeling mature HDL particles. It is likely that the PON I activity present in these HDL particles plays an important role in the anti-inflammatory effect of the newly formed HDL particles following D-4F administration in apo E null mouse.

NIELSEN, Susan S. *Poster*

PON1 variant frequencies in a population-based sample of children

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PON1 plays an important role in the detoxification of some organophosphorus (OP) insecticides, including chlorpyrifos and diazinon, which have been common in children's environments. Although young children are potentially more susceptible to OP toxicity than older individuals, perhaps in part because young children have lower PON1 levels, few studies have examined PON1 polymorphisms in relation to childhood disease. In addition, most reports of PON1 variant frequencies have used non-random samples of adults. To characterize PON1 Q192R and –108C/T genotypes in a population-based group of children, we collected dried blood spots of 100 randomly selected infants born in 1980-81 or 1990-91, from the newborn screening archives in Washington State. In this largely Caucasian population sample, 82% had a 192Q allele (allele frequency 0.62). At the –108 locus, 68% had a T allele (allele frequency 0.42). The 192Q and –108T alleles were less frequent in African American, Asian and Hispanic children. PON1 192 and –108 were both in Hardy-Weinberg equilibrium, and not in significant linkage disequilibrium. Our observations are generally consistent with most prior investigations of these genotypes. Eight (8%) children were QQ_{192}/TT_{-108} homozygotes. Previous reports suggest that young children with the QQ_{192} genotype might be particularly susceptible to chlorpyrifos toxicity, as the Q isoform hydrolyzes its oxon less efficiently than the R isoform. The TT_{-108} genotype may confer greater sensitivity to both chlorpyrifos and diazinon, in as much as the T allele is associated with lower PON1 levels. Thus, epidemiologic studies of childhood conditions possibly related to insecticide exposure may benefit from measurement of subjects' PON1 genes (and activity if serum was prospectively collected), particularly when the degree to which each child has been exposed to chlorpyrifos and diazinon is assessed.

O'LEARY, Karen A. *Poster*

Polymorphic variation in PON1 affects the detoxification of diazoxon in human serum

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Diazinon is the only organophosphate (OP) pesticide approved for use in sheep dip in the UK. It is converted to its active intermediate diazoxon through P450 (largely CYP2C19)-mediated oxidative desulphuration. Detoxification occurs through the activity of paraoxonase (PON1). Two polymorphic sites in *PON1* lead to structural changes in the protein at codons 55 (leucine (L) to methionine (M)) and 192 (glutamine (Q) to arginine (R)). The effect of these polymorphisms on diazoxon hydrolysis is presently unclear. We have investigated the relationship between these polymorphisms and serum PON1 activity towards diazoxon in 85 healthy volunteers (51 female and 34 male) of mixed ethnic origin. Serum PON1 activity was assessed using physiological conditions at pH 7.4, 150 mM NaCl and 37°C with a relatively low concentration of diazoxon as substrate (50 μM) and HPLC detection of the product, pyrimidinol. PON1 genotypes were determined by PCR and restriction enzyme digestion of the PCR products. Allele frequencies were similar to those reported previously. A wide variation in activity was found between individuals (4.6 - 39.9 nmol/min/ml serum). For PON1 192, individuals with the RR genotype had a mean activity (±SD) of 21.3 ± 9.0 nmol/min/ml serum (n=17). Activity was slightly reduced in those with the QR genotype (15.8 ± 5.7 nmol/min/ml serum, n=29, $p<0.05$) and in those with the QQ genotype, activity was reduced further, by 2 fold to 12.5 ± 6.0 nmol/min/ml serum, (n=39), $p<0.001$. For PON1 55, individuals with the LL genotype had a mean activity of 18.3 ± 8.0 nmol/min/ml serum (n=45). In comparison, activity was reduced 2 fold (12.8 ± 4.3 nmol/min/ml serum, n=37, $p<0.005$) in those with the LM genotype and 3 fold (6.3 ± 1.5 nmol/min/ml serum, n=3, $p<0.005$) in those with the MM genotype. In conclusion, although there is a wide variation in PON1 enzyme activity in individuals both within and between genotypes, those with a combination of Q and M alleles generally have a lower ability to detoxify diazoxon, which implies a potentially greater susceptibility to toxicity from diazinon.

PARAGH, Gyorgy *Selected Poster Presentation, Saturday, April 24, 2004, 2:10 – 2:15 PM*

Effect of extended-release fluvastatin on serum paraoxonase activity

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New study data suggest that clinical outcome improve with more aggressive LDL-C lowering. New formulations of fluvastatin extended-release preparations, are achieving greater reductions in LDL-C levels. The HDL-associated paraoxonase (PON) activity may play an important role in the inhibition of LDL oxidation. Previous studies have demonstrated that serum paraoxonase activity was decreased in patients with hyperlipidemia and coronary heart disease.

The aim of this study was to investigate the effect of extended-release fluvastatin (80 mg/day) on serum lipids and paraoxonase activity. 25 (11 males and 14 females) hyperlipidemic patients with Fredrickson type II.a hyperlipoproteinaemia (mean age: 59.56±9.01 yr, mean BMI: 28.33±3.58 kg/m^2) were enrolled. Serum lipids were measured and serum PON activity was determined before and after 2 and 6 months of treatment. Liver enzymes (GOT, GPT, GGT) and kidney function were unchanged.

Extended-release fluvastatin (XL) treatment significantly decreased serum cholesterol, LDL-C and apoB ($p<0.0001$) levels after 2 months, and with an additional significant ($p<0.05$) Cholesterin and LDL-C level reduction after 6 months therapy. There were no significant changes in the triglyceride, HDL-C and ApoA1 levels. Serum paraoxonase activity (120.43±66.22; 132.11±75.65 U/l; $p<0.001$) was significantly increased after 2 month fluvastatin (XL) treatment and increased further (143.95±84.54 U/l) after 6 months of therapy.

Fluvastatin XL 80 mg once daily was well tolerated and effectively managed plasma lipid profiles and improved antioxidant status by increasing serum paraoxonase activity in these patients.

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PETRIKOVICS, Ilona *Selected Poster Presentation, Friday, April 23, 2004, 5:15 – 5:20*

Recombinant Organophosphorus Acid Anhydrolase (OPH, Paraoxonase) as an Active Therapeutic Agent Derived from Nanotechnology.

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Recombinant OPH enzyme attached to the nano-delivery systems, sterically stabilized liposomes and a poly-(2-ethoxyloxazoline) dentritic polymers, was investigated as organophosphorus (OP) antidotes to protect against OP intoxication. This enzyme was obtained from *Flavobacterium sp.* and was expressed in *Escherichia coli.* It has a broad substrate specificity, which includes paraoxon, parathion, soman, sarin, diisopropylfluorophosphate and other OP compounds. The nanoencapsulated OPH was administered to mice either alone or in combination with pralidoxime (2-PAM) and/or atropine. This antidotal combination antagonizes the lethal effects of paraoxon resulting in a therapeutic increase in the LD_{50} of greater than 150 times. The therapeutic antidotal protection was not as effective as the prophylactic antagonism, where over $1000LD_{50}$ protection was obtained. Protection of free enzyme and encapsulated enzyme was compared and the encapsulated enzyme was found to persist longer and possess much greater efficacy. A reduction in the serum cholinesterase inhibition was also observed with this enhanced protection. The classic antidotal combination, 2-PAM and atropine, only blocks the pharmacological effects and reactivates the OP inhibited enzyme, while the OP agent absorbed into the body is actually degraded by the encapsulated OPH. These therapeutic antidotal systems provide by far the most effective protection against paraoxon intoxication. The nanoencapsulation technology could serve as a platform technology to deliver and to activate other protein based therapeutic agents.

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REDDY, Srinivasa *Invited Presentation, Friday, April 23, 2004, 2:05 – 2:30 PM*

Attempts to Define the Biologic Function of Paraoxonases

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Abstract: Paraoxonases are a group of enzymes with established properties and enzyme activities that provide relief from toxic environmental chemicals as well as physiological oxidative stress. My laboratory is interested in understanding the physiological function and role of paraoxonases (especially PON2 and PON3) in Atherosclerosis and Inflammation. I will discuss the expression and regulation of paraoxanases both *in vitro and in vivo.*

Biographical Sketch:

B.Sc. (Honors): Chemistry, Department of Chemistry, Osmania University, Hyderabad, India (1984),

M.Sc. (Honors): Biochemistry and Molecular Biology, Department of Biosciences and Biotechnology, University of Roorkee, Roorkee, India (1986),

Ph.D: Comparative Biochemistry, University of California at Berkeley (1992)

1987-1992: University of California, Berkeley (laboratory of Dr. Mina J. Bissell). Interdepartmental Program in Comparative Biochemistry.

1992-1997: Postdoctoral Researcher - Laboratory of Structural Biology and Molecular Medicine, School of Medicine, University of California Los Angeles.

1997-1998: Assistant Researcher - Joint appointment in the Department of Medicine and the Laboratory of Structural Biology and Molecular Medicine, School of Medicine, University of California Los Angeles.

1999-Present: Assistant Professor, Division of Cardiology, Department of Medicine, University of California Los Angeles.

2000-Present: Assistant Professor, Department of Molecular and Medical Pharmacology, School of Medicine, University of California Los Angeles.

The effects of paraoxonase on cholesterol lowering by simvastatin and atorvastatin.

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Background. LDL cholesterol lowering with HMG-CoA reductase inhibitors (statins) is an efficient treatment to reduce the cardiovascular disease risk. However, there is a significant inter-individual variation in cholesterol lowering efficiency among patients. This variability among patients may be a consequence of the pharmacokinetic profiles of statins. In the body statins are present as lactone metabolites and as ß-hydroxy metabolites. The lactone form is more efficiently eliminated from the body. Paraoxonase type 1 and type 3 PON-1 and -3) might play a crucial role in statin metabolism, because they are capable of hydrolysing lactones into the active ß -hydroxy acid form of statins and vice versa.

Plasma paraoxonase levels are highly variable among subjects, mainly due to genetic make-up. Our aim is to study the effects of paraoxonase genotype, levels and activity to cholesterol lowering in familial hypercholesterolemia patients treated with statins.

Methods. We will study the effects of six paraoxonase genotypes (PON-1 Q192R, L55M, T-107C, G-162A, G-824A and C-907G), paraoxonase levels and paraoxonase activity towards phenylacetate (PON-1 specific) and dihydrocoumarin (PON-1 and PON-3 specific) on the efficiency of cholesterol lowering by either simvastatin and atorvastatin treatment in 302 patients with familial hypercholesterolaemia. During two years of follow up, LDL- and HDL-cholesterol levels have been determined every two months.

Results. LDL levels decreased with 48.9% and HDL levels increased with 13.3% during two year treatment with statins. The 192R variant of the Q192R polymorphism predicted a more pronounced LDL-cholesterol reduction than the Q192 variant (QQ:-3.87 mmol/L; QR: -4.0 mmol/L and RR: -4.48 mmol/L, P for trend < 0.05). Furthermore, high PON-1 levels were predictive of a significant more pronounced LDL reduction during the first 8 weeks of treatment ($P<0.05$).

Conclusions. These results indicate that paraoxonase modifies the process of lipid lowering with statins.

HDL-associated PON1 Enhances HDL-mediated Cholesterol Efflux from Macrophages via the ABCA1 Transporter : a Possible Role for Lysophosphatidylcholine

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We have recently shown that serum PON1activity is inversely related to atherosclerotic lesion development and macrophage foam cell formation. Macrophage cholesterol accumulation is determined by the balance between cellular cholesterol influx and efflux. As serum PON1 is HDL-associated, we questioned whether PON1 contributes to HDL-mediated macrophage cholesterol efflux. For this purpose, we have used HDLs from PON1- knockout mice (HDL-PON1^0), from human PON1-transgenic mice (HDL-PON1Tg), and from control C57BL6 mice. Cholesterol efflux from mouse peritoneal macrophages (MPM) or from J774 A.1 cell line was found to be significantly reduced (by ~ 40%) on using HDL-PON1 ^{0}, compared to control HDL. In contrast, HDL-PON1Tg, demonstrated a 20% increased capability to stimulate cholesterol efflux as compared to control HDL. Active PON1 was required for stimulation of cholesterol efflux, since upon adding EDTA or a specific PON1 inhibitor (PD-11612) to human HDL, paraoxonase activity was reduced by up to 65%, and HDL-mediated cholesterol efflux was decreased by up to 38%. Similarly, enrichment of human HDL with purified PON1 resulted in a 10 fold increment in PON1 arylesterase activity which was associated with an 85% increased HDL-mediated cholesterol efflux. The PON1 free sulfhydryl group (Cys 284), but not the PON1 N-terminal leader sequence was needed for PON1 stimulation of HDL-mediated cholesterol efflux, as on using recombinant mutant no stimulation of cholesterol efflux could be found.

As increased HDL binding to the cells can stimulate HDL-mediated cholesterol efflux, we next studied the possible role of PON1 in HDL binding to macrophages. Indeed, the binding of HDL-PON1Tg to macrophages was increased by up to 50%, compared to the binding of HDL-PON1^0. Furthermore, addition of purified PON1 to HDL-PON1^0, resulted in a significant PON1 dose-dependent increase (by up to 51%) in HDL binding to the macrophages. Not only PON1- enriched HDL demonstrated increased binding and cholesterol efflux from macrophages, but also PON1-enriched macrophages showed similar stimulatory effects.

We have recently demonstrated that PON1 esterase action on macrophages can lead to the formation of lysophosphatidylcholine (LPC). Upon incubation of macrophages with HDL –PON1Tg, but not with HDL-PON1^0 , cellular LPC content increased by 7.7. LPC enrichment of macrophages resulted in up to 60% increased HDL binding to the cells, and up to 41% increased HDL-mediated cholesterol efflux.

On using a cAMP analog to increase the ABCA1 transporter expression and a rabbit anti-mouse SR-BI specific antibody to block the SR-BI receptor, PON1 stimulation of HDL binding and HDL-mediated cholesterol efflux from macrophages was found to involve the ABCA1 transporter.

In conclusion then, active PON1 in HDL significantly increases the lipoprotein ability to induce cholesterol efflux from macrophages secondary to PON1 stimulation of HDL binding to the cells. This effect may be related to PON1 action on cellular phospholipids, leading to the formation of LPC. Intervention means to increase serum PON1 activity can increase cholesterol efflux from macrophages, and hence- attenuate atherosclerosis development.

SERES, Ildiko *Selected Poster Presentation, Saturday, April 24, 2004, 10:05 – 10:10 AM*

Study of factors influencing the decreased paraoxonase activity with aging

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Paraoxonase (PON1) is principally complexed to HDL and is responsible, at least in part, for its antioxidant properties. PON1 activity decreases in several pathologies associated with atherosclerosis. The aim of this study was to investigate the PON1 activity and factors influencing its activity as a function of age. One hundred and twenty nine healthy subjects aged between 22 and 89 years were recruited for the study. We found that serum PON1 activity significantly decreased with age ($r = -0.38$, $p < 0.0001$) while its arylesterase activity as well as its concentration in the serum did not change significantly. HDL concentrations remained unchanged with age, however, Apo A1 concentration showed a slight negative but significant correlation with age ($r = -0.19$, $p < 0.027$). Moreover, the total cholesterol concentration was positively and significantly correlated with age ($r = 0.40$, $p < 0.001$). Thus, our results suggest that the decrease in PON1 activity can not be explained by the decrease in Apo A1 concentrations with age. HDL from elderly subjects was more susceptible to oxidation than HDL from young subjects measured by higher lipid peroxidation rate. Thus, the decrease in PON1 activity may contribute to this increased susceptibility of HDL to oxidation with aging. Altogether our results suggest that the decrease in PON1 activity may be related to the development of oxidative stress conditions with aging and the increased HDL susceptibility to oxidation in elderly subjects.

SHAMIR, Raanan *Selected Poster Presentation, Friday, April 23, 2004, 3:10 – 3:15 PM*

Intestinal paraoxonases. Possible role against oxidative stress

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Background: The paraoxonase (PON) gene family contains three genes that are believed to be involved in the protection against oxidative stress. PON1 and PON3 are highly expressed in liver and are associated in serum with high density lipoprotein (HDL), whereas PON2 has a more ubiquitous tissue expression, but is not detectable in serum. The aim of the present study was to evaluate the expression of the various PON's in the intestine, and their possible role in oxidative stress.

Methods: PON 1,2,3 mRNA expression was studied in small intestinal and colon biopsies from human subjects, and in two intestinal cell lines (Caco-2 and HT29) using semi quantitative RT-PCR. In Caco-2 and HT29 intestinal cell lines, PON 1,2,3 mRNA expression, and various PON activities (paraoxonase for PON 1, arylesterase, lactonase for PON 2, and statinase for PON 3) were also studied under oxidative stress (250μM ferrous-ascorbate).

Results: In humans, only PON 2 mRNA was present in biopsies from the small intestine and from colon. PON 1,2,3 mRNA were present in both CaCo-2 and in HT29 cell lines. Paraoxonase, arylesterase, statinase, and lactonase activities were demonstrated. Oxidation of CaCo-2 cells with iron ascorbate had no effect on the PON's mRNA, whereas in HT29 it increased PON2 mRNA expression by 264% and the expression of PON 1 and PON 3 by 26% and 42% respectively. Oxidation of CaCo-2 cells increased aryl esterase activity by 34% (from 0.47±0.13 units/mg cell protein to 0.63±0.08, p=0.03), and lactonase activity by 60.4% (from 0.94±0.13 units/mg cell protein to 1.51±0.19, p=0.0001) but not paraoxonase or statinase activities. Oxidation of HT29 increased lactonase activity by 308% (from 0.27±0.05 units/mg cell protein to 0.84±0.07, p=0.00035).

Conclusions: This is the first report demonstrating the presence of all three PON's mRNA and activities in human intestinal cell lines. Furthermore, the present study suggest that PON's are involved in the protection against oxidative stress in the intestine, and that PON 2 is mostly affected by oxidative stress.

DIANA SHIH, Ph.D. *Invited Presentation, Saturday, April 24, 2004, 11:10 – 11:35 AM*

The functions of PON1 and PON3: studies of transgenic mouse models

Diana Shih
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Abstract: Serum paraoxonase (PON1), a HDL enzyme, inhibits LDL oxidation, and in human studies, low PON1 activity is associated with atherosclerosis. To evaluate whether increased PON1 levels would attenuate atherogenesis, we generated human PON1 transgenic(Tg) mice. Plasma PON1 activities of PON1Tg mice on an atherogenic diet were 3.8- fold higher than wild-type mice. Atherosclerotic lesion sizes of the PON1Tg mice were 57% smaller than the controls. When crossed onto the apolipoprotein E knockout background, PON1Tg mice also exhibited significantly smaller lesion sizes as compared to the controls. HDL isolated from the transgenic mice prevented LDL oxidation more effectively. Our results indicate that elevated PON1 level protects against atherosclerosis. Another member of the PON family, PON3, has also been shown to inhibit LDL oxidation *in vitro*. In an attempt to test the role of PON3 in atherosclerosis, human PON3 transgenic mice were produced. These transgenic mice carried a 40 kb human genomic DNA fragment containing the PON3 gene and 4 kb of 5'-flanking and 0.4 kb of 3'-flanking sequences. The human PON3 transgene was expressed primarily in the liver, exhibiting a similar expression pattern as the endogenous mouse Pon3 gene. The human PON3 mRNA as well as the mouse PON3 mRNA were also detected in the peritoneal macrophages isolated from the transgenic mice. Studies are underway to determine whether overexpression of PON3 in transgenic mice protects against LDL oxidation and atherosclerosis.

Biographical Sketch:
Dr. Diana M. Shih obtained her B.S. and M.S. degrees from Department of Zoology, National Taiwan University, Taipei, Taiwan in 1986. She went on to Department of Zoology, University of Maryland, College Park, MD and obtained her Ph.D. degree in 1992. Dr. Shih received her postdoctoral training in the laboratory of Dr. Aldons J. Lusis, Division of Cardiology, UCLA, Los Angeles, CA, between 1993 and 1995. She joined the research faculty of Division of Cardiology, UCLA in 1996. Dr. Shih has been an adjunct assistant professor in the Division of Cardiology, UCLA since 2002. Dr. Shih has been interested in using transgenic and knockout mouse models to study the functions of PON family proteins.

Paraoxonase 2 expression is upregulated via an NADPH oxidase-dependent mechanism during monocytes differentiation into macrophages

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Paraoxonase 2 (PON2) is a member of the paraoxonases gene family. PON2 is ubiquitously present in cells, including macrophages, and it was shown to protect against cellular oxidative stress. Our aim was to analyze mechanisms involved in PON2 expression during monocyte/macrophage differentiation. PON2 expression was analyzed in vitro in THP-1 cells differentiated with 1α-25–Dihydroxyvitamin D3 (VD3), and in vivo in mouse peritoneal macrophages (MPM) isolated at increasing time intervals after intraperitoneal thioglycollate injection. PON2 mRNA expression and activity gradually increased during monocyte/macrophage differentiation, up-to 7 fold and 8 fold in vitro and in vivo, respectively. This effect was associated with a gradual increase in cellular superoxide anion production. Supplementation of vitamin E to Balb/C mice inhibited the NADPH oxidase-dependent increase in cellular superoxide anion production by 50%, and down-regulated PON2 mRNA expression and activity by 30% and by 60%, respectively. Furthermore, PON2 expression was lower by 8.9 fold in MPM isolated from $P47^{phox-/-}$, in comparison to MPM from control mice. PON2 expression was found to be regulated, at least in part, by the transcription factor AP-1, as evidenced by decreased JDP2 (AP-1 repressor) protein expression in the nucleus, and by decreased PON2 expression in presence of a JNK inhibitor (SP600125).

The present study demonstrates, for the first time, that PON2 expression increases in monocytes during their maturation into macrophage as a result of NADPH oxidase activation, and this process is partly regulated by the transcription factor AP-1. PON2 stimulation may represent a compensatory mechanism against the increase in cellular superoxide anion production and atherogenesis.

SINAN, Selma *Poster*

Differential effects of some drugs on serum and liver paraoxonase activity in mouse

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Paraxonase (aryldialkylphosphate) [E.C. 3.1.8.1] (PON1) is a calcium dependent serum esterase that is synthesised by the liver and exhibits broad substrate specificity. It catalyses the hydrolysis of organophosphates, organophosphinates, unsaturated aliphatic esters, aromatic carboxylic acid esters, and possibly carbamates. Therefore, it plays an important role in the metabolism of many xenobiotic compounds; therefore, they have a important physiological role.

However, several investigations reported that the enzyme is inhibited by some substances including especially transient metals and aliphatic alcohols. In this study, effects of magnesium sulfate and commonly used antibiotics, namely cefamezin, ampisid and genta, on mouse blood serum and liver paraoxonase activity were investigated *in vivo*. Serum and liver paraoxonase activity was determined spectrofotometricallay using paraoxan as a substrate according to a method of Gan et al and Gil et al respectively. For these studies, a group of nine mice (25g±2) were selected for intramuscular administiration of each drug. A group of three mice were included in the study for a control group, which is not subject to drug administration. Blood and liver samples were collected from each mouse at 2h, 4h and 6h after injection. For each drug, a mean of the serum and liver paraxonase activity were determined and compared to the control groups. A mean of the paraxonase activity of control group was 200 U. We found that there is a dramatic decrease on the paraxonase enzyme activity for the cefamysin-administrated mice. The most inhibition period on the enzyme activity was found at 2h following injection. The other drugs also inhibited the enzyme activity in various degrees.

This study was carried out at Balikesir University, Research Centre of Applied Sciences (Butam), Turkey.

SKLAN, Ella H. *Selected Poster Presentation, Friday, April 23, 2004, 3:05 – 3:10 PM*

Anxiety Scores in the HERITAGE Family Study Associate with Expression Variabilities and Polymorphisms in the Acetylcholinesterase / Paraoxonase Locus

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Anxiety involves complex, incompletely understood interactions of genomic, environmental and experience-derived factors and is currently being measured by psychological criteria. Here, we report previously non-perceived interrelationships between expression variations and nucleotide polymorphisms of the chromosome 7q21-22 acetylcholinesterase-paraoxonase (ACHE-PON1) locus with the trait and state anxiety measures of 451 healthy subjects from the HERITAGE Family Study. The AChE protein controls the termination of the stress-enhanced acetylcholine signaling, whereas the PON protein displays peroxidase-like activity, thus protecting blood proteins from oxidative stress damages. Inverse, reciprocal associations with both the transient score of state anxiety and the susceptibility score of trait anxiety were found for serum AChE and PON enzyme activities, corrected for demographic parameters, supporting the notion of corresponding gene expression relationships. Parallel polymorphisms in the ACHE and PON1 genes displayed apparent associations with both trait and state anxiety scores. Our findings indicate that a significant source of anxiety feelings involves inherited and acquired parameters of acetylcholine regulation that can be readily quantified, providing an independent tool for assessing anxiety measures

SOUKHAREV, Serguei

Selected Poster Presentation, Friday, April 23, 2004, 9:45 – 9:50 AM

A fluorogenic substrate for detection of organophosphatase activity.

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A new fluorogenic substrate has been designed and evaluated for the specific detection of organophosphatase (OPase) activity. Our results indicate that 7-diethylphospho-6,8-difluor-4-methylumbelliferyl (DEPFMU) is hydrolyzed specifically by the OPases mammalian serum paraoxonase and bacterial organophosphorus hydrolase (OPH). The apparent Km of DEPFMU is 29 μM for OPH, and 91 μM and 200 μM for PON1 $\underline{L_{55}R_{192}}$ and PON1 $\underline{L_{55}Q_{192}}$ isoforms of human paraoxonase, respectively. DEPFMU based assay systems are 10-100 times more sensitive for OPH and mammalian paraoxonase detection than existing methods. Importantly, DEPFMU is poorly hydrolyzed by both serum and cellular phosphatases and, therefore, may be used as part of a robust and sensitive assay for detecting not only purified, but also highly impure, preparations of OPase such as blood samples. The superior sensitivity of DEPFMU makes it potentially useful in the search for new enzymes that may hydrolyze nerve poisons such as sarin, soman and VX, monitoring the decontamination of OPs by OPH, as well as determining serum paraoxonase activity which appears to be important for protection against atherosclerosis, sepsis and OP toxicity.

SUSSMAN, Joel *Invited Presentation, Friday, April 23, 2004, 11:15 – 11:40 AM*
TAWFIK, Dan

The 3D-Structure, Mechanism and Evolution of Serum Paraoxonases

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Abstract: Despite their wide distribution and importance, the structure and mechanism of serum paraoxonases (PONs) were thus far unknown. Members of the PON family have been identified in mammals and other vertebrates, as well as in invertebrates. PONs exhibit a wide range of physiologically important hydrolytic activities, including drug metabolism and detoxification of nerve gases. PON1 and PON3 reside on HDL (the "good cholesterol") and are involved in the prevention of atherosclerosis. We describe the first crystal structure of a PON family member, a directly-evolved variant of PON1, at 2.2Å resolution. PON1 is a 6-bladed □-propeller with a unique active-site lid, which is also involved in HDL binding. The 3D structure and directed–evolution studies permitted a detailed description of PON1's active site and catalytic mechanism, and the routes by which PON family members diverged towards different substrate and reaction selectivities.

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1988 (B.Sc.)	The Hebrew University of Jerusalem; Chemistry and Biochemistry.
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1996 (Ph.D.)	The Weizmann Institute of Science, Rehovot, Israel.
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1997 - 2001	Senior Research Fellow at Sidney Sussex College and the MRC Centre for Protein Engineering, Cambridge.
1999-2000	Group Leader in Chemical Biology – The MRC Centre for Protein Engineering, Cambridge.
April 2001 - Present	Senior Scientist at the Department of Biological Chemistry, the Weizmann Institute of Science.

Research interests:

Evolution and mechanism of enzymes

Biographical sketch Joel L. Sussman

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Academic positions

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1984 – 1985	Head, Dept of Structural Chem, Weizmann Institute of Sci
1988 – 1989	Head, Kimmelman Center for Biomolecular Structure & Assembly, Weizmann Institute of Sci
1980 - 1992	Associate Prof., Dept of Structural Chem, Weizmann Institute of Sci
1992 -	Professor, Dept of Structural Biology, Weizmann Institute of Sci
1994 – 1999	Head, Protein Data Bank, Brookhaven National Laboratory, Upton, NY
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Research interests:
3D structure/function of proteins from the nervous system, 'natively unfolded proteins', protein adaptation to extreme environments; protein evolution

Purified human serum PON1 does not protect LDL against oxidation in the in vitro assays initiated with copper or AAPH

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We have optimized the method for purifying human PON1 from serum by replacing the previously used Biorad biogel anion exchange support with the micro-prep support and replacing the detergent tergitol NP-10 with *n*-dodecyl-α-D-maltoside. The new anion exchange step was superior to the old one in separating PON1 from PON3 and the platelet activating-factor acetylhydrolase (PAF-AH), as measured by 2-thio platelet activating-factor acetylhydrolase activity. Antioxidant activity of the fractions throughout the anion exchange chromatography steps was followed by determining the increase in the lag time and/or a decrease in peroxides in the copper and AAPH initiated LDL oxidation assays. The antioxidant activity did not co-purify with PON1 using either the new or the old method. In the copper initiated assays the activity in the fractions could largely be accounted for by the "antioxidant" activity of the detergent. We then examined the antioxidant activity of purified human serum PON1 preparations that had been stored at 4°C for 4-10 years. All these preparations exhibited significant antioxidant activity in the copper initiated assay, but this did not correlate with the amount of detergent, protein, arylesterase activity, statinase activity or PAF-AH activity in the samples. Overnight dialysis of the old preparations resulted in a 30 to 40 % loss of their arylesterase activities and a complete loss of their antioxidant activities. When one of the old preparations was further purified through an anion exchange chromatography step, using the old method, the antioxidant activity did not bind to the support whereas the PON1 was almost completely bound. In conclusion, the above improved purification procedure should provide a preparation of PON1 that is more suitable for biochemical studies. The previously reported antioxidant activities of purified PON1 in the *in vitro* LDL oxidation assays appear to be due to some contaminant(s), possibly leeching from the polypropylene storage tubes or of microbial origin, which is present in older preparations. The inability of freshly purified PON1 to protect LDL from oxidation suggests that the removal of PON1 from its natural environment may impair its antioxidative activity or that this assay may be an inappropriate method to elucidate the mechanism(s) of PON1's anti-atherogenic properties.

Oxidative stress induces expression of paraoxonase 2 (PON2)

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We have previously shown that PON2 plays a role in the reduction of intracellular or local oxidative stress, but little if any, is known about the regulation of PON2 gene expression. To determine the regulation of PON2 expression in mouse models of oxidative stress, real-time PCR was used to quantify PON2 message levels in the livers of C57BL6 (12 week old, n=10 per group) and apoE null (8 week old, n=10 per group) female mice fed either a chow, high-fat, or atherogenic diet (15 weeks for C57BL6 mice and 8 weeks for apoE null mice). PON2 mRNA levels, represented as a ratio of PON2/GAPDH expression, were elevated in C57BL6 mice fed an atherogenic diet compared to C57BL6 mice fed either a chow diet or high-fat diet (chow vs. atherogenic: 1.0 +/- 0.27 vs. 1.7 +/- 0.66, $p<0.05$ and high-fat vs. atherogenic: 1.4 +/- 0.42 vs. 2.2 +/- 0.73, $p<0.05$). PON2 expression was not significantly different in comparing C57BL6 mice fed a chow and high-fat diet. In apoE null mice, PON2 mRNA was higher in mice fed either a high-fat diet or atherogenic diet compared to mice fed a chow diet (chow vs. high-fat: 1.2 +/- 0.24 vs. 1.8 +/- 0.56, $p<0.005$ and chow vs. atherogenic 1.4 +/- 0.57 vs. 2.3 +/- 0.55, $p<0.005$). PON2 expression was not significantly different when comparing apoE null mice fed an atherogenic diet and high-fat diet. Interestingly, the regulation of PON2 gene expression is quite different from the other two members of the PON family, PON1 and PON3. In the same set of mouse studies described above, PON1 expression was significantly repressed in response to both a high-fat diet or atherogenic diet in C57BL6 mice, whereas PON3 expression was not altered by either a high-fat diet or atherogenic diet in both C57BL6 and apoE null mice. To determine the key factors that play a role in the regulation of PON2 gene expression, we examined the effect of cholic acid (a key ingredient of the atherogenic diet), and TNF-□ and IL-6 (pro-inflammatory cytokines which are upregulated in oxidative stress and atherosclerosis), on PON2 expression in J774 mouse macrophages. Chenodeoxycholic acid (CDCA; 100 □M) treatment induced PON2 mRNA by almost 3 fold after 6 hours of stimulation in J774 cells. Addition of TNF-□ (10 ng/ml) and IL-6 (10 ng/ml) also induced PON2 message to a maximum of 3 fold over untreated cells after 6 hrs of stimulation, and 2 fold over untreated cells after 4 hours stimulation, respectively. PON2 gene expression returned to basal levels seen in untreated J774 cells by 24 hours following stimulation by all the three ligands. Our results suggest that PON2 gene expression, similar to other intracellular antioxidants, is positively regulated in response to oxidative stress and pro-inflammatory stimuli. We are currently studying the response elements in the PON2 promoter that participate in the regulation of PON2 expression by oxidative stress, CDCA and inflammatory cytokines.

Functional Polymorphisms and Triglycerides Are Independent Determinants of Pon1 Enzymatic Activity in Pregnant Women

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PON1 is an enzyme found in HDL that detoxifies oxidized lipids in HDL and LDL. It also exhibits an arylesterase activity on organophosphates. The *PON1* gene contains five common polymorphisms, three in the promoter [-162, -108 (in linkage disequilibrium with -909)] and two in the coding region (M55L, Q192R) with varying but incomplete linkage disequilibrium. M55L and -108 affect arylesterase activity, and Q192R affects organophosphate substrate specificity.

Many studies have associated *PON1* polymorphisms with cardiovascular diseases. Our previous studies demonstrated that functional polymorphisms in PON1 were strongly associated with enzymatic activity in both pregnant women [26-30 weeks gestation] and neonates [Chen, J., et al., Environ. Health Persp. 111, 1403, 2003]. In a previous report on maternal PON1 activity, maternal lipids and birth weight, average PON1 activities and serum triglycerides were found to increase during pregnancy, to be highly correlated to one another, and to correlate negatively with birth weight [Roy, A.C., et al., Gynecol. Obstet. Invest. 38, 10, 1994].

In our study of three ethnicities (N = 402), we were not able to confirm any relationship between PON1 activity or *PON1* genes and birth weight. Lipid profiles [HDL, LDL, cholesterol and triglycerides] were determined for a subset of 117 Caucasian and Hispanic mothers. There was a trend toward higher lipid values in all categories with increasing PON1 activity, approaching the level of significance for cholesterol [p(trend) = 0.08 for PON1 tertiles; p = 0.05 with low PON1 tertile reference value] but highly significant for triglycerides [p(trend) = 0.0008; p = 0.005 with low PON1 tertile reference value]. PON1 polymorphisms explained 20% of the PON1 variability, while triglycerides explained 5%; collectively, polymorphisms and triglycerides explained 25.4%. The contributions appear to be additive, suggesting triglycerides are an independent determinant of PON1 activity.

YEUNG, David T. ***Selected Poster Presentation, Friday, April 23, 2004, 11:45 – 11:50 AM***

Structure/Function Analyses of Human Paraoxonase-1 Mutants

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The level of serum paraoxonase1 (PON1) in circulation has been reported to correlate with sensitivity to organophosphorus (OP) poisons, suggesting that PON1 acts as an *in vivo* bioscavenger to afford some protection against highly toxic OP poisons to include nerve agents. *In vitro* analyses of human PON1 (huPON1) have shown the enzyme catalyzes the hydrolysis of a variety of OP nerve agents. Clearly, however, the catalytic activity and serum concentration of huPON1 are insufficient to protect humans against OP toxicity. Prior efforts in our laboratory have shown that site directed mutagenesis can enhance the catalytic efficiency and/or structural stability of an enzyme. However, to rationally design such mutants, a three dimensional model of huPON1 is required. To date, efforts to determine the x-ray crystallographic structure of huPON1 have been unsuccessful. We are attempting to validate a proposed six-fold □-propeller structural model of the enzyme by using site directed mutagenesis to substitute amino acid residues predicted to be in structurally and catalytically critical sites. The gene encoding huPON1 was inserted into the pCDNA3 expression vector and mutated before being transiently transfected into human 293T embryonic kidney cells. The resultant mutant huPON1 proteins are being analyzed for catalytic efficiency against p-nitro phenyl acetate. Confirmation of the proposed model would allow us to design strategies for enhancing huPON1 catalytic activity against OP nerve agents. We estimate that a 10-fold increase in catalytic activity would be sufficient to provide substantial *in vivo* protection against poisoning by chemical warfare nerve agents.

BERT N. LA DU – 50 YEARS OF RESEARCH AND COUNTING....

Dr. La Du obtained his B.S. degree in Chemistry from Michigan State College, an M.D. degree from the University of Michigan Medical School and a Ph.D. degree in Biochemistry from the University of California (Berkeley). He started his studies on drug metabolism in Bernard B. Brodie's laboratory of Chemical Pharmacology at the National Heart Institute and authored the first paper on the liver microsomal enzyme system that later became known as the P-450 enzyme system. In the fifties and early sixties he held different positions at the National Heart Institute and the National Institute of Arthritis and Metabolic Diseases (Medical Director at the latter 1961-63), where he pursued studies on drug and amino acid metabolism, and identified the enzymatic deficiency in two metabolic disorders of amino acid metabolism: alkaptonuria and histidinemia. In 1963 Dr. La Du became Professor and Chairman of the Pharmacology Department at New York University, where he started his studies on cholinesterase characterizing the kinetic properties of purified variant forms of the enzyme in patients with drug sensitivity to the muscle relaxant succinylcholine. In 1974 he moved back to Ann Arbor as Chairman of the Pharmacology at the University of Michigan and continued his research on serum cholinesterase. His laboratory determined the complete sequence of the enzyme, cloned the gene and developed methods to characterize variants at the DNA level, diagnose complicated mutations and do family studies to follow the patterns of these allelic variations. Over 35 distinct variants were identified as specific mutations affecting the structure of the coding region of this enzyme. Dr. La Du's laboratory has become the international center for research on this topic. As Emeritus Professor of Pharmacology (Active), he was assigned to the Department of Anesthesiology at the University of Michigan Medical School, where he served as Associated Chair for Research and Acting Director of Research (1990-2000). In 2000 the Department of Anesthesiology endowed a Professorship in his name.

Prof. La Du has been an active member of and held representative positions at many professional organizations, e.g. Chairman of the Biochemical Division (1964-65) and President (1970) of the New York Academy of Sciences, and Chairman of the Division of Drug Metabolism (1970-72), Secretary-Treasurer (1973-74) and President (1978-79) of the American Society for Pharmacology and Experimental Therapeutics. He was an organizer and Co-Chairman of many seminal scientific meetings such as the 1st Workshop on Drug

Metabolism at NYU (1966), the 1st International Conference on Pharmacogenetics (New York Academy of Sciences, 1967), and the 1st Workshop on Immunopharmacology (NYU, 1971). He has authored more than 200 scientific papers, reviews and chapters in textbooks and monographs.

Studies on serum paraoxonase (PON1) has become another main focus of Prof. La Du's research in the last two decades. His laboratory has discovered the major PON1 polymorphism (192 Q/R) and developed a reliable method of phenotyping many years before the structural basis for it was known. Subsequently this method was used to measure allelic frequencies in several population samples including the American, French, Saudi Arabian, and Sudanese families. A method for purification of human PON1, developed in his lab in the early 90-ies, is still the main one used today, and Dr. La Du had provided purified enzyme to many other laboratories worldwide. His lab has demonstrated by expression of PON1 that both arylesterase and paraoxonase activities are catalyzed by the same protein. His lab has discovered also that in human and mice paraoxonase is one member of a gene family along with other members now called PONs 2 and 3. PONs from other species (rabbit, dog, chicken, turkey, fish *Danio regio*, frog and the worm *C. elegans*) were also cloned and sequenced. Unlike PON1, PON2 and PON3 do not hydrolyze organophosphate compounds, but all PONs do share lactonase activity, which was also discovered in his laboratory. The lactone substrates include several statin drugs and the widely used diuretic spironolactone. His current studies are looking for the physiological functions of the PONs, their ability to prevent oxidative damage of cells and tissues, and which substrates and reactions are involved in their protective activities.

Prof. La Du has fruitful collaborations with many other laboratories working on PON in the country and abroad – with Dr. Fogelman's group at UCLA, Dr. Forte at Berkeley, Dr. Connelly at the University of Toronto, Dr. Aviram at the Rambam Medical Center, Haifa, Israel, to name just few. He has mentored numerous graduate students and post-docs. His over 30 paraoxonase papers were cited more than 1500 times. His nice personality and life of dedication to science, make it simple for his colleagues and students not just respect, but love him.

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